BL-27B/2021G539

Radio-adaptive response induced by the targeted cytoplasmic irradiation in normal human fibroblasts with X-ray microbeams via bystander effects by gap-junction mediated cell-to-cell communication

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1 Introduction

Cellular response, such as bystander effect, for low-dose or low-fluence irradiations must be one of the major concerns for radiology or health physics. It is very important for us to understand low-dose or low-fluence radiations induced radio-biological effects via bystander effects, such as genomic instability and radio-adaptive response. Such radio-biological effects are also very essential to investigate secondary carcinogenesis after tumor radio-therapy and evaluate radiation risk such as the accident of Fukushima Daiichi Nuclear Power Plants caused by the Great East Japan Earthquake at 2011.

A microbeam-irradiation system is powerful method to examine low-dose or low-fluence radiations induced radio-biological effects and so far there are many studies available to examine cellular responses such using microbeam radiations. However, the most of them were carried out using high-LET-particle radiations and limited studies were available to use low-LET electromagnetic radiations. In this study we have planned to make clear low-dose or low-fluence radiations induced radio-biological effects by low-LET electromagnetic radiations using the X-ray microbeams produced by PF at BL-27.

We so far reported low-LET X-ray induced bystander effects in normal human fibroblasts as follows:

1) The cellular bystander effect for chromosomal damage via gap-junction mediated cell-to-cell communication was not induced in cells immediately after irradiations with both cell nucleus and cytoplasm [1].
2) The bystander cell-killing effect was induced when cells were irradiated with targeted cell nucleus alone [2].
3) The higher frequency of gene mutation at the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) locus was observed in the progeny of the following 20-cell generations from the X-ray-microbeam irradiated cells than those 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].

In this project (2021G539) we have been studying radio-adaptive responses induced in cells irradiated with targeted cytoplasm beforehand and then irradiated with targeted cell nuclear by X-ray microbeams. The results suggest that when the cells were irradiated the cytoplasm beforehand, the cell survival in cells irradiated with the targeted cell nuclei was returned to the control level, suggesting the radio-adaptive response [5-10]. This year we examined intercellular radio-adaptive response using the gap-junction inhibitor.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell No.: RCB0222, Cell name : NB1RGB,) were used for the target cells. 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. Cell nuclei were stained by Hoechst 33342 and captured by the computerized-cell-irradiation system.

X-ray-microbeam irradiations were carried out using monochromatic X-ray (5.35keV) at BL-27B. Targeted cytoplasmic irradiations with the X-ray microbeams were performed using the microbeam-cell-irradiation system [5]. Briefly, we made the microbeam collimating the beam size of 30μm x 30μm in which the center of the microbeam the gold-made mask that was 22 μm in diameter and 20 μm in height on a thin SiN film was set in order to shield the nucleus from X-ray exposure. When targeted cell nuclei irradiations were carried out, we used the X-ray microbeams collimating the beam size of 10μm x 10μm. The irradiation dose was 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 captured cells were irradiated with X-ray microbeams beforehand and then targeted cell nuclei of 100% cells were irradiated at the 180min interval within

which the cells were kept in a CO_2 incubator at 37°C after the first cytoplasmic irradiation. A specific inhibitor of gap-junction mediated cell-to-cell communication (40 μM of gamma-hexachlorocyclohexane) was added to the culture medium 1hr before the first cytoplasmic irradiation to the second cell nucleus irradiation in order to examine the effects of bystander responses via gap-junction mediated cell-to-cell communication. Cell-killing effect was detected 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 surviving cell.

3 Results and Discussion

This year we carried out the repeat experiments using independent beam times for 100% of targeted nuclear irradiation (10R=0.092Gy) alone with the gap-junction inhibitor. The result was shown in Fig.1. The cell survival irradiated with all cell nuclei alone was 79% and no cell-killing effect was observed in the cytoplasmic irradiation alone. The cell survivals were rising to 95-98% when either 10% or 100% of cytoplasm was irradiated beforehand. When treating with the gap-junction inhibitor, the cell survivals for targeted nuclear irradiation were around 72% with the presence or absence of the targeted cytoplasmic irradiation beforehand. The results clearly showed that the bystander effect via intercellular response with gap-junction mediated cell-to-cell communication plays an essential role of reducing cell-killing effect, suggesting radio-adaptive response.

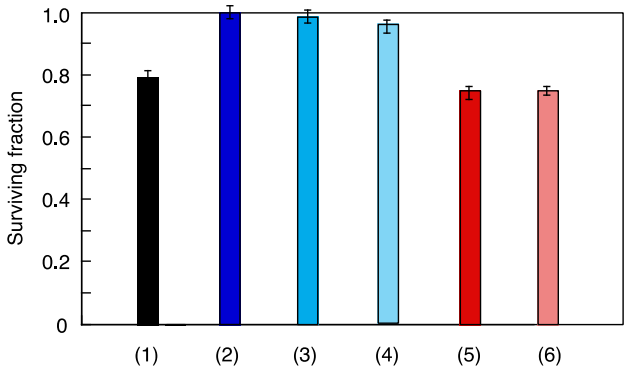

Fig.1: Cellular lethal effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation of monochromatic X-ray microbeams. (1) 100% of targeted nuclear irradiation (10R=0.092Gy) alone, (2) 100% of targeted cytoplasmic irradiation (0.092Gy) alone, (3) 100% of targeted 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), (5) 100% of targeted nuclear irradiation (10R=0.092Gy) alone with the gap-junction inhibitor, (6) Randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy) with gap-junction inhibitor. The data showed the average and the standard error of at least 8 independent experiments.

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