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The effect of hydroxyl radical for radio-adaptive-response induction by the bystander effect through secreted factor(s)

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1 Introduction

Radiobiological effects can be induced by either direct or indirect ionizing action caused by depositing energy into cells and/or water molecules from radiation. The central paradigm implies that radiation actions only affect the directly irradiated cells by radiation and/or radicals, and that non-irradiated cells do not contribute to radiobiological consequences. This paradigm is the basis for the current system of risk estimation of radiobiological effects. However, the paradigm has recently been challenged by the progress of non-targeted cellular responses, such as bystander effects, for low-dose or low-fluence radiations and it must be one of the major concerns for radiology or health physics. It is very essential for us to understand radiobiological effects induced by low-dose or low-fluence-radiations, and cellular responses, such as bystander effects, play an important role to elucidate the mechanism of the radiation actions. Bystander effects should be fundamental to investigate cellular responses, such as genomic instability or radio-adaptive response, and its mechanism must contribute to reveal secondary carcinogenesis after tumour radio-therapy and evaluate radiation risk for effect on human body such as the accident of Fukushima Daiichi Nuclear Power Plants caused by the Great East Japan Earthquake in 2011.

Communication of signalling events from direct irradiated cells to non-irradiated cells is one of major concerns for bystander effects. Secreted factors are one of the strong pathways for bystander effects and previous studies demonstrated that medium from irradiated cells could induce biological effects in non-irradiated cells, when transferred medium from irradiated cells to non-irradiated cells. Mothersill and Seymour reported the first result that a highly significant biological response in cell-killing effect in non-irradiated both normal and malignant cells that received medium from ^{60}Co-gamma-ray irradiated cells [1]. The result suggested against the mechanism of the bystander effect that irradiated cells secreted unknown factor(s) in the culture medium, which was capable of killing non-irradiated cells. They also reported the individual variation in producing such a bystander signal in medium from irradiated cell cultures using primary cultures of normal human urothelium [2].

A microbeam-irradiation system is powerful method to examine radio-biological effects induced by low-dose or low-fluence radiations 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 radio-biological effects of low-dose or low-fluence radiations irradiated with low-LET electromagnetic radiations using the X-ray microbeams.

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

1) The bystander effect for chromosomal damage through gap-junction mediated cell-to-cell communication was not induced in cells immediately after irradiations with both cell nucleus and cytoplasm [3].
2) The bystander cell-killing effect was induced when cells were irradiated with cell nucleus alone [4].
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 of non-irradiated control cells and it was reduced to the control level when treating with a specific inhibitor of gap-junction mediated cell-to-cell communication [5, 6].

We also published the manuscript regarding the bystander cellular effects by the factor(s) secreted into the culture medium from irradiated cells with high-LET radiations using the heavy-ion microbeams generated with the Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) in National Institutes for Quantum Science and Technology (QST) [7].

In this study we have been examining radio-adaptive responses induced in cells irradiated with targeted cytoplasm beforehand and then irradiated with targeted cell nucleus by X-ray microbeams. The results suggest that when the cells were irradiated the cytoplasm beforehand, the cell survival of the targeted-cell-nucleus irradiation was returned to the control level, suggesting the radio-adaptive response [8-14]. This year we focused on hydroxyl radicals (· OH) as a secreted factor produced for inducing radio-adaptive response through the bystander effect in cells irradiated with targeted cytoplasm beforehand.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell No.: RCB0222, Cell name : NB1RGB) were used for this research project. The first target of secreted factor(s) is hydroxyl radicals, which were scavenged by Dimethyl

sulfoxide (DMSO). This year we examined the effect of DMSO to taking part in the radio-adaptive response induced in cells irradiated with targeted cytoplasm beforehand and then irradiated with targeted cell nucleus by X-ray microbeams. The irradiation procedure was described elsewhere [8-10]. Briefly, 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 (10R ~ 0.092Gy) with the monochromatic X-ray microbeams (5.35keV) to the normal human fibroblasts were carried out using the cell-irradiation system making 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. The targeted cytoplasm randomly selected 10% of all cells detected in the microbeam-irradiation dish were irradiated with X rays (10R ~ 0.092Gy) beforehand and then targeted cell nucleus of 100% cells were irradiated with X rays (10R ~ 0.092Gy) with collimating the beam size of 10μm x 10μm at the 180min interval within which the cells were kept in a CO_2 incubator at 37°C after the first cytoplasmic irradiation. The cells were treated with 1% DMSO form the cytoplasmic irradiation to the end of the nucleus irradiation. Cell-killing effect was detected with a colony-forming assay as a reproductive cell death. After finishing the nucleus irradiation, cells were trypsinized and a defined number of cells plated onto 100mm plastic dish to make 60-70 colonies per dish. The colonies were 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.

Results and Discussion

The result was shown in Fig.1. The first we examined the effect of DMSO for cell-killing effect by hydroxyl radicals. The cell survivals were 76% for nucleus irradiation alone and 91% for nucleus irradiation with DMSO. The data suggested that hydroxyl radicals were produced and led to cell death partly with the irradiation of 5.35keV-X-ray microbeams.

The cell survivals were rising to 95% when randomly selected 10% of cytoplasm was irradiated beforehand (10R ~ 0.092Gy). The data suggested that radio-adaptive response was induced by the cytoplasmic irradiation. When treating with the DMSO, the cell survival was around 98% and no change was observed between presence and absence of DMSO. The result clearly showed that induced radio-adaptive response could not be suppressed by DMSO.

The final step we will plan to examine the effect of secreted factor(s) scavenged by ascorbic acid (vitamin C), which will be possible radical-like molecules as a secreted factor of the bystander effect by the cytoplasmic irradiation of X-ray-microbeams.

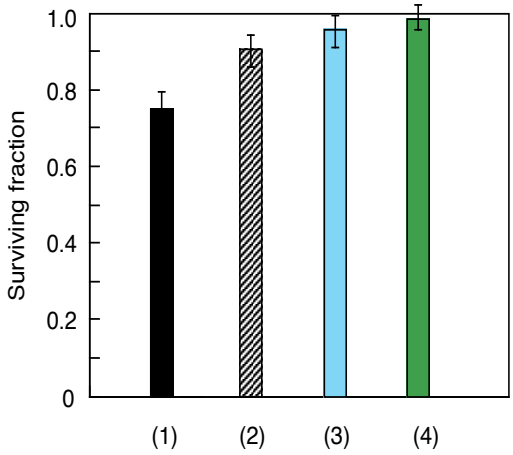

Fig.1: The effect of DMSO for cell-killing effects in normal human fibroblasts irradiated with X-ray microbeams. (1) 100% of targeted nuclear irradiation (10R ~ 0.092Gy) alone, (2) 100% of targeted nuclear irradiation (10R ~ 0.092Gy) with 1% DMSO, (3) Randomly selected 10% of targeted cytoplasmic irradiation (10R ~ 0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (10R ~ 0.092Gy), (4) Randomly selected 10% of targeted cytoplasmic irradiation (10R ~ 0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (10R ~ 0.092Gy) with 1% DMSO

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