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# Do the secreted factors from X-ray-microbeam irradiated cells affect radio-adaptive-response induction through bystander effects?

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## 1 Introduction

Direct energy deposition into cells and/or water molecules from radiation can induce either direct or indirect ionization through the action of hydroxyl radicals and elicit radiobiological effects. This central paradigm in radiology or health physics implies that the radiobiological consequences only affect the directly irradiated cells by radiation and/or radicals, and that non-irradiated cells do not contribute to the radiobiological effects induced through radiation exposure. The paradigm is the basis for the current system of risk estimation of radiobiological effects. However, the paradigm has recently been challenged by the discovery of non-targeted cellular responses, such as bystander effects for low-dose or low-fluence irradiations and it 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- radiation induced radio-biological effects through bystander effects, such as genomic instability and radio-adaptive response. Such radio-biological effects are also very essential to investigate secondary carcinogenesis after tumour 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.

Regarding the bystander effects induced by secreted factor(s), previous studies demonstrated that medium from irradiated cells could induce increased biological effects in non-irradiated bystander 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].

In general there are many studies available to examine radiation-induced bystander effects. However, the most of them were carried out using high-linear-energy-transfer (high-LET)-particle radiations and limited studies were available to use low-LET electromagnetic radiations. In this research project we have planned to make clear low-dose or low-fluence-radiation induced radio-biological effects by low-LET electromagnetic radiations using the X-ray microbeams produced at BL-27B.

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 [3].
2) The bystander cell-killing effect was induced when cells were irradiated with targeted 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 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 [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 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 [8-14]. This year we focused on secreted

factor(s) produced from X-ray-microbeam irradiated cells 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 study. The first target of secreted factor(s) is the radical, which were scavenged by Dimethyl sulfoxide (DMSO). This year we first checked the toxicity of DMSO against normal human fibroblasts. 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, one day before the treatment of DMSO. Cell nuclei were stained by Hoechst 33342 and added 0.128M DMSO for 3 hours, which condition was the same with X-ray-microbeam irradiation. Cell-killing effect was detected with a colony-forming assay as a reproductive cell death. After 3-hour treatment of DMSO at room temperature, 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

Figure 1 shows photos of the colonies either control medium without any treatment or medium treated with 0.128M DMSO. It is observed no difference in the number of colonies in the dish, the shape and size of each colony in cells treated with either normal medium or DMSO medium.

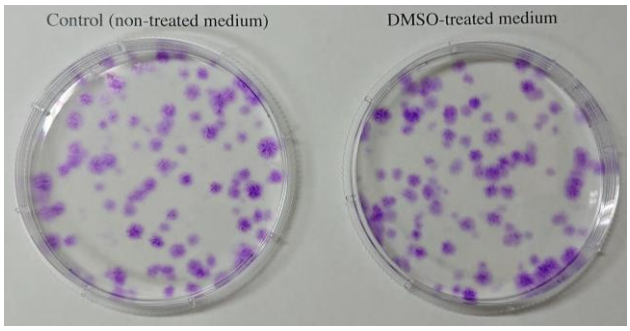


Fig.1: The results of the colony-formation assay for non-treated control medium (the left-hand dish) and DMSO-treated medium (the right-hand dish). Each colony was formed for the 16-day incubation in a $CO_2$ incubator at 37°C.

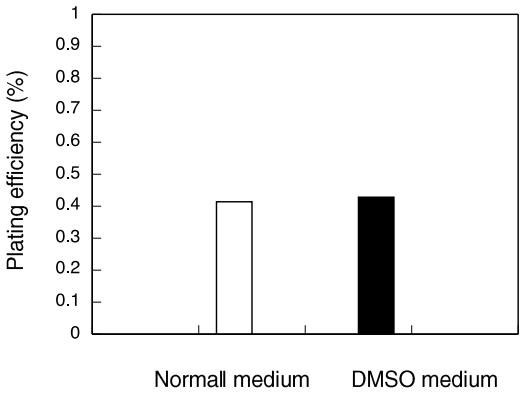


Fig.2: The plating efficiency of NB1RGB cells treated with either normal medium or DMSO medium.

The plating efficiency, which is one of the indicators for cell viability, was 41.4% for normal medium and 42.8% for DMSO-treated medium (figure 2). The result clearly indicated that there was no toxicity for our cell sample treated with 0.128M DMSO.

The next step we will examine the effect of secreted factor(s), which were scavenged by DMSO, for observed radio-adaptive response through bystander effect using the cytoplasmic irradiation of X-ray-microbeam irradiations.

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