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Dose-Dependent Chromosomal Damage via Bystander Effect in Normal Human Fibroblasts Induced by Monochromatic X-ray Microbeams

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1 Introduction

Most studies for radiation-induced bystander effect have been carried out using the microbeam and/or broadbeam irradiations with high linear energy transfer (LET) particle radiations and only limited data is available to understand bystander effects induced by low-LET electromagnetic radiations, such as X or gamma rays. However, the study for low-LET-radiation induced bystander effects can surely provide the important implications for evaluating risk such a low-dose (rate) exposure as the accident of Fukushima Daiichi Nuclear Power Plants.

In this study we have been studying low-LET-radiation induced bystander cellular effects using X-ray microbeams. This year, we examined dose-dependent chromosomal aberrations via bystander effect to clarify mechanism(s) of radiation-induced bystander effects, extending to the last year's experiment.

2 Experiment

Early passaged (passage number 6-10) normal human skin fibroblasts obtained from the Riken BioResource Center were irradiated with the monochromatic 5.35-keV X-ray microbeams collimated with 20μm x 20μm at BL27B. Irradiations were carried out with 0.19, 0.38, 0.76 and 0.95Gy in each point using the 256 cross-stripe method described previously [1, 2]. Thirty minutes before irradiation, half of the dishes were treated with 18-α-glycyrrhetinic acid (AGA), which is a specific inhibitor of gap-junction mediated cell-cell communication. At the irradiation period, cultures were confluent and allowed direct intercellular communication via the gap junction. We examined micronucleus (MN) formation as the indicators for chromosomal aberrations. The MN formation was detected using the cytokinesis block technique. Briefly, after irradiation cells were subcultured and allowed to grow in the presence of 2 μg/ml cyctochalasin B for 72 h incubation at 37°C. Then the cells were fixed in ethanol and stained with Hoechst 33342 solution. At least 500 cells were examined for each data point under a fluorescence microscope and only micronuclei in binucleated cells were scored as a damaged cell.

3 Results and Discussion

Figure 1 shows the dose-response formation of MN induced by X-ray microbeams. There observed no significant difference between the absence and presence of AGA and the percent of the binucleated cells with MN was increasing to 2-4% as a dose-dependent manner. The results shown were beyond our expectation, because we can estimate that only 0.04% of total cells on the dish was irradiated directly with the X-ray microbeams by our 256 cross-stripe method [1, 2]. If MN formation could occur only in directly irradiated cells, the percent of MN formation could never go above 0.04%, assuming no bystander effects. There is evidence that the bystander effect via secreted factor(s) from the irradiated cells and/or medium, not gap-junction mediated cell-cell communication, plays an important role in inducing chromosomal aberrations in the case of low-LET X rays.

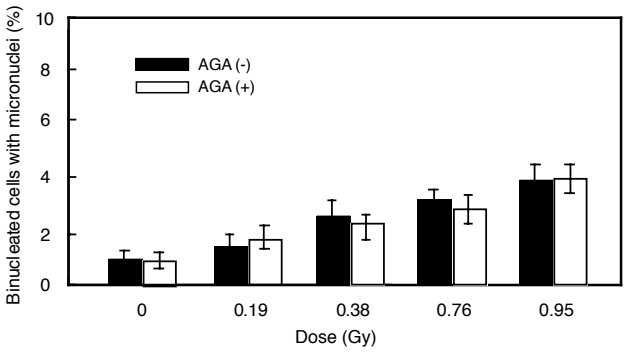

Fig.1: The Dose-dependent formation of MN in normal human fibroblasts irradiated with X-ray microbeams. Closed bars show MN formation with the absence of AGA and open bars show with the presence of AGA. The results were the means and standard deviations from the 3 independent beam times.

Acknowledgements

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References

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