BL-27B/2011G572

Chromosomal Aberrations via Bystander Effect in Normal Human Fibroblasts Irradiated with Monochromatic X-ray Microbeams

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1 Introduction

The traditional dogma of radiobiological effects recently has been challenged by the observation of bystander effect, which is that similar effects can also be induced in non directly irradiated cells neighboring on irradiated cells. Most studies for bystander effect have been carried out using microbeam/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. In this study we have been examining bystander cellular effects and demonstrated the bystander lethal effect by the targeted cell nucleus irradiations last year [1]. This year, we examined chromosomal aberrations to understand mechanism(s) of inducing bystander effect in chromosome/DNA levels.

2 Experiment

Monochromatic 5.35-keV X-ray microbeams with 20μm x 20μm size were produced with the cell irradiation system at BL27B. Irradiations were carried out with 40R in each point using the 256 cross-stripe method described previously [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 MN formation induced by X-ray microbeams, comparing to neon-ion microbeams at the Takasaki Ion Accelerators for Advanced radiation Application of the Japan Atomic Energy Agency. The MN formation by neon-ion microbeams in the absence of AGA was higher than that in the presence of AGA, while we observed no significant difference between the absence and absence of AGA by X-ray microbeams. The percent of the binucleated cells with MN was 3-6% in the absence of AGA, whereas still exist in 2-4% level in the presence of AGA with any of the radiation sources. We estimate that only 0.04% of total cells on the dish was irradiated directly with the microbeams by our 256 cross-stripe method [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. However, the present results showed beyond our expectation. There is clear evidence that bystander effect occurred in the induction of chromosomal aberrations. The difference in our data between X-ray- and neon-ion microbeams suggests that two different mechanisms play a critical role in inducing bystander effect, such as gap-junction mediated cell-cell communication in the absence of AGA and secreted factor(s) to culture medium from the irradiated cells in the presence of AGA. And the bystander effect via secreted factor(s) only induces by low-LET X rays.

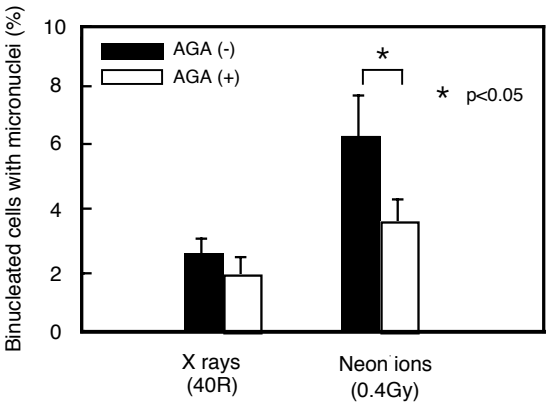

Fig.1: The MN formation irradiated with X-ray and neon-ion microbeams. The results were the means and standard deviations from the 3 independent beam times.

Acknowledgements

This study was supported in part by grants from the Japan Society of the Promotion of Sciences (23・01513) and the Quantum Beam Technology Program from the Japan Science and Technology Agency.

References

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