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The Cellular Response of DNA Damage in X-irradiated Cancer Cell Population -Verification of Radiation-Induced Field Size Effect in Cancer Cell-

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1 Introduction

Microbeam radiotherapy (MRT) based on the cellular response to non-uniform radiation has attracted attention as a new radiotherapy [1]. However, the mechanism underlying the effectiveness of MRT is largely unknown.

X-ray microbeam is very effective for investigating the response of cells to non-uniform radiation. Previously, we investigated the response of DNA damage in normal human cell populations non-uniformly irradiated with X-ray microbeams of different beam sizes, and found that even at the same dose, the smaller the X-irradiated field size on the cell population, the less DNA double strand breaks per cell in X-irradiated cells [2]. These results indicate that X-irradiated cells received some signal (rescue signal) from surrounding nonirradiated cells, and DNA damage was rapidly repaired or cells with DNA damage were eliminated. This radiation-induced field size effect (RIFSE) was thought to be related to the effectiveness of MRT on normal tissues.

In this study, to clarify the effectiveness of MRT on cancer tissues, we investigated the response of cancer human cell populations irradiated with different beam sizes to DNA damage.

2 Experiment

Cell culture: Human lung adenocarcinoma cells, A549, (JCRB cell bank, japan) were cultured on a cover glass with a grid line (GC1300, Matsunami Glass Industry Co., Ltd.) in MEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2 to prepare a cell population of about 5×10^4 cells in 530 mm^2. The irradiation field can be specified by using the cover glass with a grid line.

X-ray microbeam irradiation: X-ray microbeam was delivered by an X-ray microbeam generator at BL-27B with 5.3 keV. Dose rate was 20 R/s.

The response of cell population to DNA damages: In order to analyze the difference in cell population response depending on the X-irradiation size to DNA damage, the size of 0.02 mm^2, 0.09 mm^2, 0.81 mm^2, and 1.89 mm^2 on the cell population were irradiated with X-ray of 1 Gy. Subsequently, cells were incubated for 4 h at 37°C under 5% CO_2. After incubation, DSBs were detected by 53 binding protein 1 (53BP1) immunofluorescent staining, and the numbers of DSB per cell were analyzed .

Examination of radiation-induced rescue effect (RIRE) to cell survival in cancer cells: A549 cells were X-irradiated with 10 Gy and co-cultured with non-irradiated cells using cell culture inserts for 48 h at 37°C under 5% CO_2. Subsequently, survival rate was calculated by counting the number of living cells.

3 Results and Discussion

Table1. The response of cancer cell population to DNA damages.

	No. of 53BP1 per cell ± SD
	4h
Control	0.07± 0.3
0.02 mm^2	1.7 ± 1.4
0.09 mm^2	1.4 ± 1.2
0.81 mm^2	1.5 ± 1.4
1.89 mm^2	1.4 ± 1.3

Table2. The examination of RIRE to cell survival in cancer cell.

	Survival rate± SD (%)
Control	98.8 ± 0.7
X-irradiated cells without co-culture	57.0 ± 15.8
X-irradiated cells cocultured with non-irradiated cells	44.1 ± 7.2

I. No change in the number of DNA damages depending on the size of the X-ray-irradiated cell population was observed with A549 (Table.1).
II. No improvement in survival rate was seen in X-irradiated cells cocultured with non-irradiated cells. (Table. 2).

From the results of this study, we found that RIRE-mediated RIFSE may not occur in cancer cells and seemed to be related to the effectiveness of MRT on cancer tissue in contrast to normal tissue.

References

[1] Kobayashi, K. *et al*., *Int. Congr. Ser.* **1258** (2003).
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