BL-27B/2019G030

The Response of Cells to DNA Damage and Cell Proliferation Depends on the Size of the X-irradiated Cell Population

Mitsuaki OJIMA[1]*, Atsushi ITO[2], Keiji SUZUKI[3], Noriko USAMI[4], Maki OHARA[4], and Michiaki KAI[1]

[1] Oita University of Nursing and Health Sciences, Oita 840-1201, Japan
[2] School of Engineering, Tokai Univ., Hiratsuka, Kanagawa 259-1292, Japan
[3]Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
[4]Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization, 1-1 Oho, Tsukuba, 305-0801, Japan

1 Introduction

After the Fukushima nuclear accident, internal exposure due to insoluble radioactive cesium (Cs) deposited in the lungs has become a problem [1, 2]. Although the health effects of internal exposure from radioactive Cs is very important issue from the perspective of radiation protection [3, 4], the biological mechanism has not been clarified.

X-ray microbeam is very effective to reproduce the situation of local exposure in internal exposure. In this study, we investigated the response of normal human cell populations irradiated with different beam sizes to DNA damage and cell proliferation.

2 Experiment

Cell culture: Primary normal human fibroblasts from the lung, MRC-5 (European Collection of Cell Cultures), 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 4~48 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 .

The response of cell population to cell proliferation: In order to analyze the difference in cell proliferation depending on the X-irradiation size, the size of 0.02 mm^2, 0.09 mm^2, 0.81 mm^2, and 1.89 mm^2 in the cell population were irradiated with X-ray of 1 Gy. Subsequently, cells were incubated for 24~48 h at 37°C under 5% CO_2. After incubation, cell proliferations were detected by Ki67 immunofluorescent staining, and the frequencies of Ki67 positive cells were analyzed.

3 Results and Discussion

Table1. The response of cell population to DNA damages

	No. of 53BP1 per cell ± SD		
	4h	24h	48h
Control	0.57± 0.088	0.69 ± 0.14	0.47 ± 0.039
0.02 mm^2	3.68 ± 0.64*	0.51 ± 0.043	
0.09 mm^2	3.30 ± 0.19*	0.44 ± 0.075	0.44 ± 0.087
0.81 mm^2	5.53 ± 2.29*	0.85 ± 0.19	0.67 ± 0.087*
1.89 mm^2	6.49 ± 0.72*	0.80 ± 0.17	0.88 ± 0.20*

* P<0.05, T-test

Table2. The response of cell population to cell proliferation

	Frequencies of Ki67 positive cell ± SD	
	24h	48h
Control	0.72 ± 0.068	0.29 ± 0.031
0.02 mm^2	0.61 ± 0.029	
0.09 mm^2	0.60 ± 0.038	0.19 ± 0.0058*
0.81 mm^2	0.35 ± 0.11*	0.17 ± 0.095*
1.89 mm^2	0.37 ± 0.058*	0.14 ± 0.038*

* P<0.05, T-test

I. An X-irradiated cell populations size-dependent reduction in the number of DNA damages was observed at < 0.81 mm^2 (Table.1). In addition, there was a tendency that DNA damage remained for a long time in 0.81 mm^2 and 1.89 mm^2.

II. When the size of X-irradiated cell population was larger than 0.09 mm^2, the frequency of cell proliferation decreased at 24 hours after X-irradiation (Table. 2).

These results indicate that when the X-irradiation size was small, X-irradiated cells received some signal (rescue signal) from surrounding nonirradiated cells, and DNA damage were rapidly repaired or cells with DNA damage were eliminated.

References

[1] Chino, M. *et al*., *J. Nucl. Sci. Technol*. **48** (2011).
[2] Satou, Y. *et al*., *Anthropocene* **14** (2016).
[3] Sakama, M. *et al*., *Springer, Tokyo* (2016).
[4] Ishikawa, T. *et al*., *J. Radiol. Prot*. **38** (2018).

* ojima@oita-nhs.ac.jp