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Repair Process of DNA Double Strand Breaks Induced by X-ray Bystander Effect

Mitsuaki OJIMA[1]*, Yohei KAWAMATA[2], Shogo ONIZAWA[2], Michiaki KAI[1], Noriko USAMI[3], and Atsushi ITO[2]

[1] Oita University of Nursing and Health Sciences, Oita 840-1201, Japan
[2] School of Engineering, Tokai Univ., Hiratsuka, Kanagawa 259-1292, Japan
[3] Photon Factory, KEK, Tsukuba, Ibaraki 305-0801, Japan

1 Introduction

Recent evidence suggests that damage signals may be transmitted from irradiated to non-irradiated cells in a population, leading to the induction of genetic changes that include gene mutations in bystander cells that received no radiation exposure. This phenomenon, the radiation-induced bystander effect, has been observed in mainly fibroblast and epithelial cells by assay for various endpoints, including DSB, chromosome aberrations, cell killing, neoplastic transformation, formation of micronuclei, and changes in gene expression [1-3]. In the previous study, we investigated the repair kinetics of DSB in non-irradiated primary normal human fibroblasts (MRC-5) co-cultured with 20 mGy-irradiated MRC-5. After 48 h of co-culture, 81% of the initial numbers of DSB remained in non-irradiated MRC-5 [4]. In addition, when MRC-5 were irradiated with 1000 mGy after co-cultivate with 20 mGy-irradiated MRC-5, we found that the numbers of DSB significantly decreased compared with 1000 mGy-irradiated MRC-5 which were not experienced co-cultivate with 20 mGy-irradiated MRC-5 (under preparing submission). From these previous findings, we hypothesized that DSB resulting from the radiation-induced bystander effects might not be repaired, and unrepaired DSB by radiation-induced bystander effect might contribute to induction of radioadaptive response.

In the present study, as a first step, we investigated the repair kinetics of DSB in MRC-5 which was irradiated with X-rays directly by X-ray microbeams.

2 Materials and Methods

Cell culture. Primary normal human fibroblasts from the lung, MRC-5 (European Collection of Cell Cultures), were grown on a sterilized cover glass in MEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2. All experiments were performed using non-dividing confluent cell cultures, the confluent state was kept for at least 24 h before experiment, in order to eliminate disparate cell-cycle phase radio-sensitivities.

X-ray microbeam irradiation. X-ray microbeam were delivered by an X-ray microbeam generator in Photon Factory with 5.3 keV. Dose rate was 20 R/s.

Repair kinetics of DSBs in directly X-irradiated MRC-5 by X-ray microbeam. Cover glass with confluent cells was put on a Mylar sheet. They were then irradiated with 1 Gy of X-ray microbeam in various cell population (0.3 mm × 0.3 mm, 0.9 mm × 0.9 mm, 1.2 mm × 3 mm). Control samples were sham-irradiated. Subsequently, cells were incubated for 24 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 were determined by assessing the number of 53BP1 foci.

3 Results and Discussion

The numbers of DSB in directly X-irradiated cell increased depending on size of X-irradiated cell population (Fig. 1). This result means that even if the cumulative dose of radiation is the same per cell, with the increasing number of directly X-irradiated cells, the DSB may easy to remain in directly X-irradiated cells. This phenomenon may be caused by the interaction of directly X-irradiated cells and non-irradiated bystander cells. In the next step, we will investigate whether the number of directly X-irradiated cell affect the induction of bystander effect.

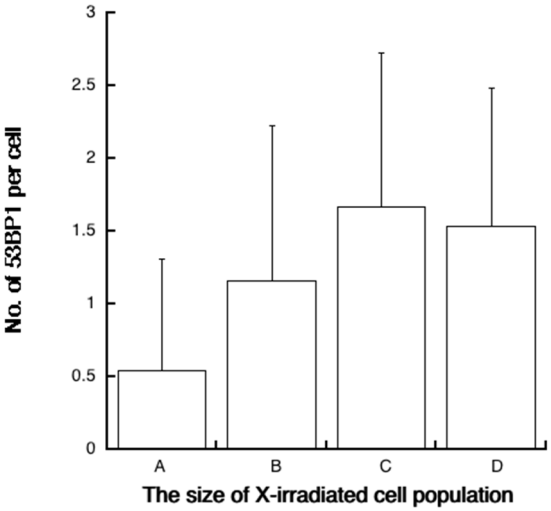

Fig. 1. Number of DSBs in X-irradiated MRC-5
A: Control, B: 0.3 mm × 0.3 mm, C: 0.9 mm × 0.9 mm, D: 1.2 mm × 3 mm

References

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* ojima@oita-nhs.ac.jp