BL-27B/2015G074

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

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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 DNA double strand breaks (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-cultivation 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-cultivation 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 the first trial to prove this hypothesis, we investigated repair kinetics of DSB in both X-irradiated MRC-5 and bystander MRC-5 using by X-ray microbeam.

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 installed at BL-27B in Photon Factory (Tsukuba, Japan) with 5.3 keV. Dose rate was 20 R/s.

Reapir kinetics of DSBs in both bystander MRC-5 and direct irradiated MRC-5. Cover glass with confluent cells was putted on a Mylar sheet. They were then irradiated with X-ray microbeams at 0.1 Gy and 1 Gy. Control samples were sham-irradiated. Subsequently, cells were incubated for 30 min, 120 min and 240 min at 37°C under 5% CO_2. After incubation, DSB was detected by both 53 binding protein 1 (53BP1) and phosphorylated ataxia telangiectasia mutated kinase (p-ATM) immunofluorescent staining.

3 Results and Discussion

The numbers of DSB in X-irradiated MRC-5 and in bystander MRC-5 were determined by assessing the number of both 53BP1 foci p-ATM foci. The dose-response relationship for the number of DSBs in X-irradiated MRC-5 was supralinear (Fig.1). However, the number of DSBs in bystander MRC-5 showed non-linear dose response curve (Fig.1). As a result of incubation to allow DSB repair, we found that the number of 53BP1 foci was decreasing slowly in X-irradiated MRC-5 until 240 min after X-irradiation at 1 Gy (Fig.2). However, in bystander MRC-5, the number of 53BP1 foci was almost constant for 240 min (Fig.2). These results suggested that the signals transmitted by X-irradiated MRC-5 at 1 Gy might be able to cause unrepaired DSB in bystander MRC-5. In the next step, we will investigate whether the radioadaptive response is observed in bystander MRC-5 with unrepaired DSB.

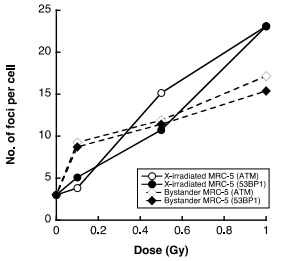

Fig.1 Dose-response curve of DSBs in X-irradiated MRC-5 and in Bystander MRC-5

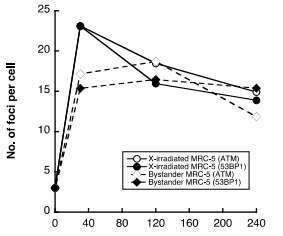

Fig.2 Repair kinetics of DSBs in X-irradiated MRC-5 and in Bystander MRC-5

References

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