

## 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 DSB, chromosome aberrations, cell killing, 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]. This results suggested that the bystander effects can increase chances of genetic changes in non-irradiated cells, and may enhance the risk of radiation carcinogenesis in the low dose region.

On another hand, it has been accumulating evidence that detrimental effects, such as DSB and cell death, in directly irradiated cells are reduced upon receiving feedback signals from non-irradiated bystander cells or from the medium previously conditioning non-irradiated bystander cells. [5]. This phenomenon is called radiation-induced rescue effect and may constitute one of the protective effects of low dose radiation.

Both the radiation-induced bystander effect and the radiation-induced rescue effects represent important phenomena that have a significant impact on novel biological responses induced by low dose radiation. However, these mechanisms remain largely unknown.

In this study, we analyzed the repair kinetics of DSB in MRC-5 which was irradiated with X-rays directly by X-ray microbeams to clarify the actual situation of radiation-induced rescue effects.

### 2 Materials and Methods

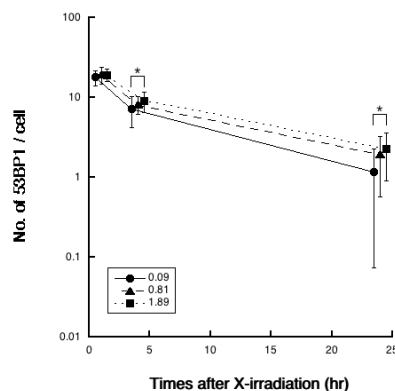
**Cell culture.** Primary normal human fibroblasts from the lung, MRC-5 (European Collection of Cell Cultures), were grown on a sterilized cover glass in MEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified incubator with 5% CO<sub>2</sub>. All experiments 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 Mirer sheet. They were then irradiated with 1 Gy of X-ray microbeam in various cell population (0.09 mm<sup>2</sup>, 0.81 mm<sup>2</sup>, 1.89 mm<sup>2</sup>). Control samples were sham-irradiated. Subsequently, cells were incubated for 24 h at 37°C under 5% CO<sub>2</sub>. 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

Depending on the size of X-irradiated cell population, the both the numbers of DSB in directly X-irradiated cell increased and the DSBs in directly X-irradiated cell persisted after X-irradiation. 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.



**Fig. 1: Number of DSBs in X-irradiated MRC-5**  
 ● : 0.09 mm<sup>2</sup>, ▲ : 0.81 mm<sup>2</sup>, ■ : 1.89 mm<sup>2</sup>

### References

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