The defect of non-homologous end joining substantially enhanced the focus formation of Rad51 after X-ray irradiation, but not after heavy-ion irradiation

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DNA double-strand breaks (DSBs) are caused by exposure of DNA to ionizing radiation, and these are repaired primarily by non-homologous end joining (NHEJ), homologous recombination (HR), or microhomology mediated end joining (MMEJ) in mammalian cells [1]. Accelerated heavy-ion particles with a high linear energy transfer (LET) induce complex clustered DNA damage, which is considered an obstacle to efficient repair and induces different biological effects compared to low-LET radiation. To analyze the repair mechanism for DSBs caused by heavy-ions, we investigated cell sensitivity to heavy-ions by using a wild-type CHO cell and two CHO mutant lines deficient in HR[2] or NHEJ[3] in the previous study[3] and observed that HR is mainly involved in the repair pathway induced by high-LET ionizing radiation[4]. However, several studies suggest that NHEJ is also involved in DSB repair caused by heavy ion[5,6], and the repair mechanism is still controversial.

In this report, we investigated the formation of Rad51 foci, which is involved in strand transfer and is essential for HR[7], using CHO cells and mutant cell line (V3) deficient in NHEJ. The number of Rad51 foci per cell in V3 cells was twice that in CHO cells 1 h after X-ray irradiation, suggesting that NHEJ and HR work competitively to some extent (Fig. 1). In contrast, the number of Rad51 foci in V3 cells was similar to that in CHO cells 1 h after C-ion irradiation (LET = 80 keV/μm), suggesting that HR works mainly in DSB repair.

The number of Rad51 foci was maximum immediately after X-ray irradiation and decreased gradually, whereas the number of Rad51 foci increased from 1 h to 8 h and then slightly decreased from 8 h to 16 h after C-ion irradiation, indicating that DSBs induced by C-ion are repaired slowly compared to those induced by X-ray. In addition, the number of Rad51 foci significantly increased in V3 cells than in CHO cells 16 h after C-ion irradiation. This result suggests that NHEJ is also involved in repair pathway. The processing of damaged DNA strands by various enzymes may enable NHEJ to repair DSBs caused by heavy-ion irradiation at a later period. As the apoptosis in CHO cells starts 18-24 h after irradiation, the results of survival may not necessarily reflect the repair process directly.

Now we are investigating the localization of 53BP1 and Rif1 after heavy-ion irradiation, which are involved in the selection of repair pathways for repairing DSB[8,9]. Our preliminary experiments showed that Rad51 foci were only observed in S-G2 cells after heavy-ion irradiation (data not shown). Therefore, we are also planning to examine how cell cycle affects repair kinetics.

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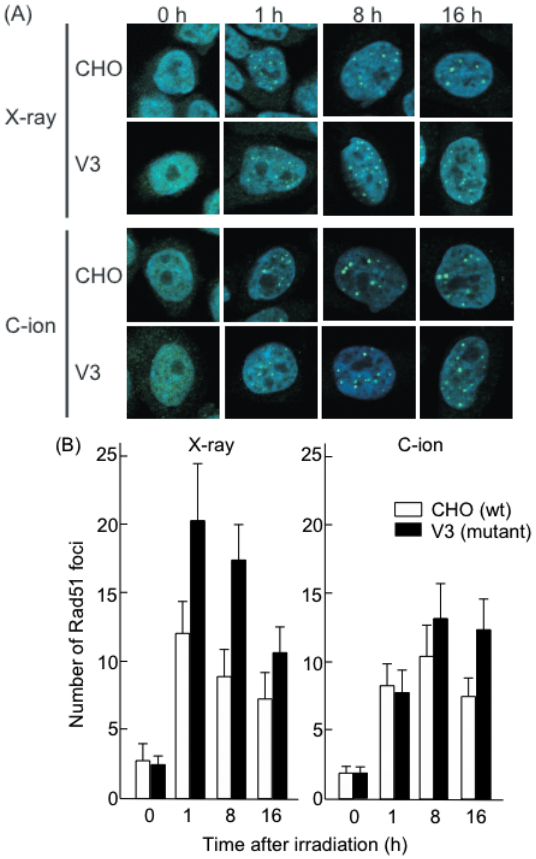

Fig. 1 (A) Representative images of CHO or V3 nuclei (blue) with Rad51 foci (green). Cells were irradiated with 5 Gy of X-ray or carbon ions and fixed with 4% paraformaldehyde at indicated time points from irradiation. Foci formation of Rad51 was detected using immunofluorescence staining. (B) Kinetics of Rad51 foci formation in CHO (open box) or V3 (closed box) cells. Rad51 foci were scored in 2D by using a Zeiss microscope with ApoTome from 1-16 h post irradiation. The average number of foci per Rad51-positive cells is shown. Error bars represent the standard deviations.

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