Non-homologous end joining is the major repair pathway for DNA double strand breaks in human fibroblast after heavy-ion irradiation

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Accelerated heavy-ion particles with high linear energy transfer (LET) induce complex clustered DNA damage, which are difficult to repair. Although the numbers of treatment facilities and patients undergoing heavy-ion therapy are increasing, the DNA repair mechanism caused by heavy-ion irradiation is not fully understood at the molecular level. DNA double-strand breaks (DSBs) are the most lethal damage caused by ionizing irradiation and are repaired primarily by non-homologous end joining (NHEJ) or homologous recombination (HR) in mammalian cells, whereas alternative NHEJ (alt-NHEJ) and/or single strand annealing (SSA) work only when both NHEJ and HR are impaired.

Several published results of survival assay using mammalian mutant cell lines deficient in NHEJ or HR suggest that NHEJ is inhibited after heavy-ion irradiation.[1,2] On the other hand, studies using inhibitors and CHO mutant cell lines suggest that NHEJ is a major repair pathway after heavy-ion irradiation, although HR is more important for higher-LET radiation.[3,4] In addition, clustered DNA damage enhances end resection, which promotes alt-NHEJ and/or SSA.[5] Therefore, the DNA repair mechanism after heavy-ion irradiation is still controversial in higher eukaryotes.

In this study, the DSB repair mechanism after heavy-ion irradiation was examined using specific inhibitors against repair proteins. Human fibroblast NB1RGB cells were irradiated with X-ray, carbon ions, or argon ions in the presence of an NHEJ inhibitor (NU7441) or HR inhibitor (B02), as illustrated in Fig. 1. The repair efficiency was estimated by the kinetics of the phosphorylated histone H2AX foci, which reflect the presence of DSBs (Fig. 2).

The number of phosphorylated histone H2AX after X-ray irradiation decreased with similar kinetics in the presence of NU7441 or B02, suggesting that both pathways work competitively and compensate each other. In contrast, the foci formation after heavy-ion irradiation in the presence of NU7441 was delayed and reached maximum 3 h after irradiation, probably because the histone H2AX is phosphorylated partly by DNA-PK. The foci number decreased by 30% within 5 h of heavy-ion irradiation with or without inhibitors. NU7441 inhibited DSB repair after heavy-ion irradiation at later time points (5–24 h), whereas B02 had no effect on DSB repair after heavy-ion irradiation. These results suggest that high-LET radiation induced complex damage with dirty broken ends as well as simple repairable damage. In addition, it is also suggested that NHEJ is the major repair pathway after heavy-ion irradiation and DNA damage checkpoint delays the HR kinetics after heavy-ion irradiation.

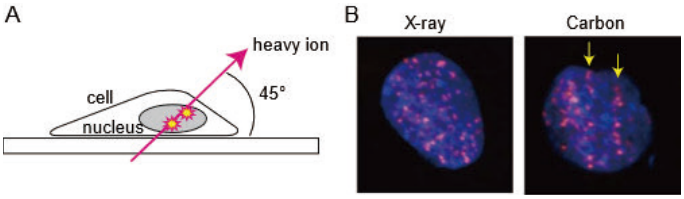

Fig. 1. A: Schematic of the irradiation. Cells were tilted at 45 degrees for heavy-ion irradiation. B: Representative immunostaining images of phosphorylated histone H2AX (red) and nuclei (blue) after 2 Gy of X-ray or carbon-ion irradiation. Arrows indicate the track of ions.

References
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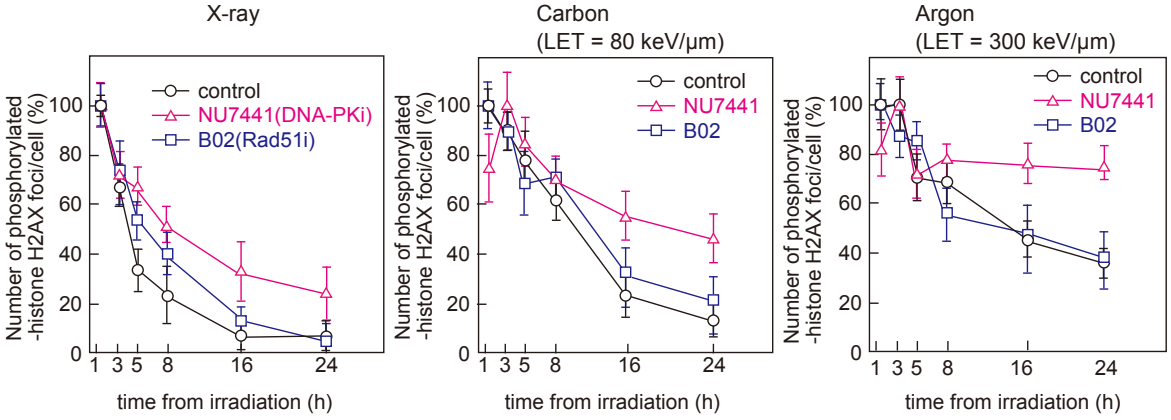

Fig. 2. Time course of phosphorylated histone H2AX foci after irradiation. NB1RGB cells were pretreated with 3 μM NU7441 (DNA-PK inhibitor) or 10 μM B02 (Rad51 inhibitor) for 3 h and irradiated with 2 Gy of X-ray, carbon ions (LET = 80 keV/μm), or argon ions (LET = 300 keV/μm). The percentage of foci per cell was plotted by normalizing the numbers at the maximum time point as 100% after irradiation.

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