Kinetics of Rad51 foci in G2 phase after heavy-ion irradiation in mammalian cells

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DNA double-strand breaks (DSBs) are the most lethal type of damage caused by ionizing irradiation and are repaired mainly by non-homologous end joining (NHEJ) or homologous recombination (HR) in mammalian cells; alternative NHEJ and/or single-strand annealing work only when both NHEJ and HR are impaired. Accelerated heavy-ion particles with high linear energy transfer (LET) induce complex and fragmented DNA damage affecting the pathway choice and the efficiency of DSB repair.

Several published results of survival assay using Chinese hamster mutant cell lines deficient in NHEJ or HR suggest that NHEJ is inhibited after heavy-ion irradiation.[1,2] In contrast, studies using inhibitors and mouse 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] It is also reported that clustered DNA damage enhances end resection, which could promote HR, alt-NHEJ, and SSA.[5,6] Therefore, the DNA repair mechanism after heavy-ion irradiation is still controversial in higher eukaryotes.

Our previous study using human fibroblast and a specific inhibitor against NHEJ or HR suggests that NHEJ and HR work competitively and compensate for each other after X-irradiation.[7] On the other hand, NHEJ is the major repair pathway after heavy-ion irradiation, and HR does not seem to compensate for NHEJ. However, it is unknown whether HR efficiently repairs DSBs caused by heavy ions or whether the DNA damage checkpoint delays the entry into the S/G2 phase, where HR occurs.

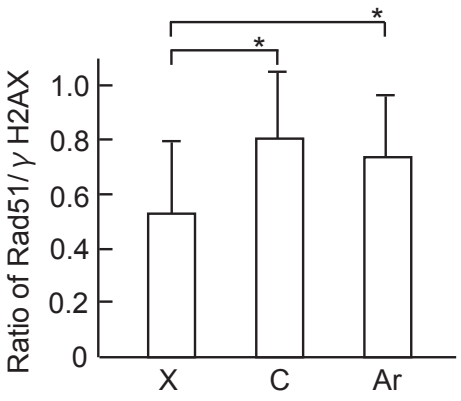

Fig. 1. Ratio of Rad51/phosphorylated histone H2AX (γH2AX) foci 1 h after irradiation in HeLa cells. Aphidicolin (10 μM) was added 30 min before irradiation, and cells were irradiated with 2 Gy of X-rays, carbon ions (LET = 80 keV/μm), or argon ions (LET = 300 keV/μm) and incubated in the presence of aphidicolin for 1 h after irradiation. The foci formation of Rad51 and γH2AX was detected by immunostaining. Student't t test: $^*P < 0.01$. Error bars represent SD.

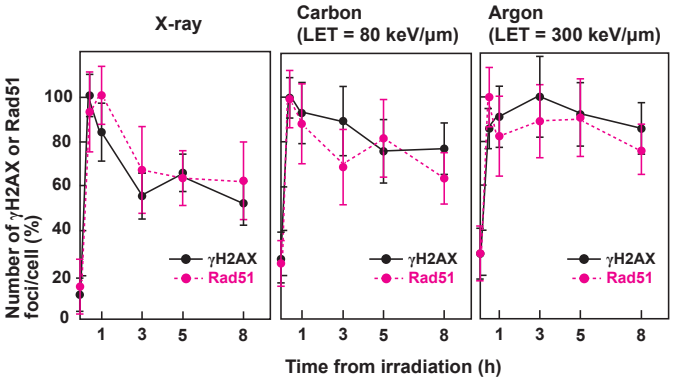

Fig. 2. Time course of phosphorylated histone H2AX (γH2AX) and Rad51 foci after irradiation in G2 cells. The percentage of foci per cell was plotted by normalizing the numbers at the maximum time point as 100% after irradiation.

In this study, we examined the DNA repair kinetics as well as the repair pathway usage in the G2 phase. Exponentially growing HeLa cells were irradiated with X-rays, carbon ions, or argon ions and incubated in the presence of aphidicolin to arrest S-phase progression and inhibit transition from the S to the G2 phase. The repair efficiency and pathway usage were estimated by the kinetics of the phosphorylated histone H2AX foci and Rad51 foci, which reflect the DSBs and HR, respectively. S-phase cells were identified by the pan-nucleic staining of the phosphorylated histone H2AX and excluded from analysis.

The ratio of Rad51/phosphorylated histone H2AX foci induced by heavy-ion irradiation was higher than that induced by X-rays (Fig. 1). The number of Rad51 foci decreased more slowly after heavy-ion irradiation than after X-ray irradiation, with the kinetics similar to those of phosphorylated histone H2AX (Fig. 2). These results suggest that HR is favored after heavy-ion irradiation, although HR repairs DSBs less efficiently after heavy-ion irradiation.

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References

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