The amount of chromatin-bound Rad51 reached maximum after 2 Gy heavy-ion irradiation in mammalian cells

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Accelerated heavy ions with high linear energy transfer (LET) are known to induce complex clustered DNA damages that are difficult to repair and result in higher cell-killing. Although the number of treatment facilities and patients undergoing heavy-ion therapy are increasing, the DNA repair mechanism underlying heavy-ion irradiation is not fully understood at the molecular level.

Among DNA damages caused by ionizing radiation, DNA double strand breaks (DSBs) are the most lethal. Mammalian cells have four pathways to repair DSBs: non-homologous end joining (NHEJ), homologous recombination (HR), alternative NHEJ (alt-NHEJ), and single strand annealing (SSA). After exposure to low-LET radiation, NHEJ is the dominant repair pathway throughout the cell cycle, whereas HR works only in the late S/G2 phase. Alt-NHEJ and SSA are considered to be functional only when both the NHEJ and HR are inactive.[1)]

Previous studies show that DSBs caused by high-LET radiation promote end-resection, which could lead to resection-dependent HR, alt-NHEJ, and SSA.[2)] Therefore, we have focused on the localization of Rad51, which is an essential protein for HR, after heavy-ion irradiation. Our preliminary study shows that the chromatin recruitment of Rad51 increases up to 5 Gy in a dose-dependent manner, but is suppressed by high dose (>15 Gy) heavy-ion irradiation.[3)] However, it is unknown whether the suppression of Rad51 recruitment is due to the limiting factors for chromatin loading, such as 53BP1 and RNF168[4)] or the checkpoint control to arrest the cell cycle prior to the late S/G2 phase. This is because we used unsynchronized cells. In fact, a recent study has shown that high-LET activates the ATR-Chk1 pathway, which regulates the S-phase checkpoint and can modify the HR kinetics.[5)]

In this study, we examined the Rad51 recruitment onto chromatin as well as the phosphorylation of DNA-PK using the cells synchronized at the late S/G2 phase to exclude the effects of the retarded S-phase progression. HeLa cells were synchronized at the G1/S boundary by double thymidine block. Then, cells were released into the S-phase and irradiated with argon ions (LET = 300 keV/μm) of different doses (0.5–30 Gy) at 7 hours from the G1/S boundary (Fig. 1). At this time, half of the cell population was in late S phase and the other half was in the G2 phase. The chromatin fractions were obtained at 1 hour after irradiation and subjected to immunoblotting (Fig. 2(A)). The amount of phosphorylated DNA-PK increased approximately linearly up to 30 Gy, whereas that of Rad51 increased linearly and reached maximum at 2 Gy (Fig. 2(B)).

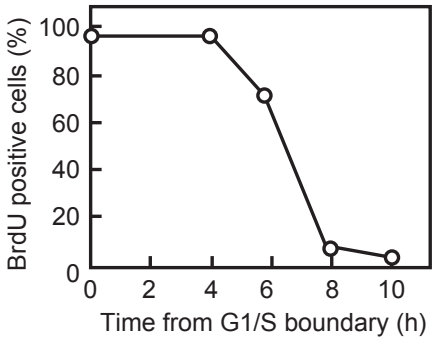

Fig. 1. S-phase progression from the G1/S boundary. HeLa cells synchronized at the G1/S boundary were released into the S phase. S-phase progression was monitored by pulse-labeling with bromodeoxyuridine (BrdU) and immunofluorescence staining.

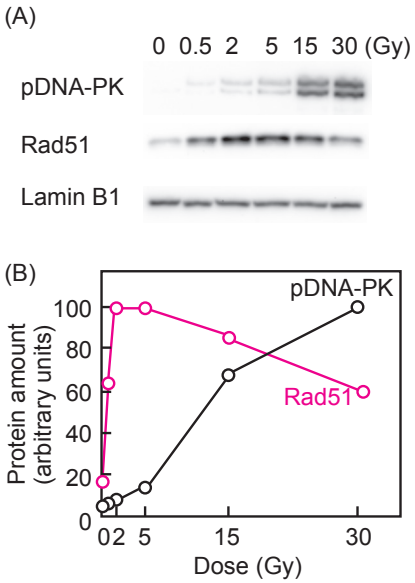

Fig. 2. Immunoblot analysis of chromatin-bound repair proteins after irradiation. (A) HeLa cells synchronized in late S/G2 phase were irradiated with argon ions. Triton-insoluble fractions (chromatin fractions) were prepared at 1 hour following irradiation and subjected to immunoblotting. Phosphorylated DNA-PK (pDNA-PK) and Rad51 were detected as markers for NHEJ and HR, respectively. Lamin B1 was detected as a loading control. (B) The relative intensities of phosphorylated DNA-PK and Rad51 were measured using luminoimage analyzer and normalized against the intensity of lamin B1.

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The amount of Rad51 decreased gradually at higher doses (>5 Gy). These results suggest that HR is suppressed at doses higher than 2 Gy and SSA or alt-NHEJ is promoted in the late S/G2 phase. Currently, we are investigating the time course of Rad51 recruitment in late S/G2 cells after irradiation as well as the chromatin recruitment of SSA/alt-NHEJ factors.

References

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