Recruitment of Rad51 onto chromatin is suppressed by high dose heavy-ion irradiation in mammalian cells

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Among the DNA damages caused by an ionizing radiation, DNA double strand breaks (DSBs) are the most lethal because accumulation of misrepaired or unrepaired DSBs can lead to a loss of genetic information and cell death. Mammalian cells have four pathways to repair DSBs: canonical non-homologous end joining (NHEJ), homologous recombination (HR), alternative NHEJ (alt-NHEJ), and single-strand annealing (SSA). Recently, DNA repair pathways are considered as targets for cancer therapy, because their inhibitors increase the efficacy of radiotherapy. It is also important to know whether error-prone pathways such as SSA and alt-NHEJ are involved in DSB repair, to estimate the risk of secondary carcinogenesis in radiotherapy.

After exposure to low-linear energy transfer (LET) radiation such as X-ray, NHEJ is the dominant repair pathway throughout the cell cycle in mammalian cells, whereas HR can only repair DSBs in the late S/G2 phase. Additionally, alt-NHEJ or SSA is effective when both NHEJ and HR are impaired, and it contributes to genome rearrangements and oncogenic transformations. Previous studies have suggested that the end-resection of DSBs is stimulated after heavy-ion irradiation throughout the cell cycle,[1,2] which can promote HR, SSA, or alt-NHEJ. However, the repair mechanism after heavy-ion irradiation has not been fully understood.

Our previous study using mammalian cells and specific inhibitors against NHEJ or HR suggested that NHEJ is the major repair pathway after 2 Gy heavy-ion irradiation.[3] We also showed that HR is favored after heavy-ion irradiation in the G2-phase; however, the DSB repair by HR is less efficiently than that after X-ray irradiation.[4]

Recent studies revealed that the number of DSBs is an additional key parameter of pathway selection because several repair proteins limit HR at high doses in mammalian cells.[5,6] In this study, we examined the amount of Rad51 on chromatin after irradiation, to investigate the effect of irradiation dose on the repair pathway selection. Rad51 is an essential core component of HR and recruited around DSBs after irradiation. Exponentially growing HeLa cells were irradiated with argon ions (LET = 300 keV/μm) of different doses (0.5–15 Gy), and chromatin fractions were obtained and subjected to immunoblot analysis (Fig. 1(A)). The amount of Rad51 in the chromatin fraction increased up to 5 Gy in a dose-dependent manner (Fig. 1(B)). However, the amount of Rad51 decreased following 15 Gy irradiation. We performed the same experiment using a carbon-ion beam (LET = 80 keV/μm), which yielded similar results (data not shown). These results suggest that HR is suppressed after high dose irradiation (∼15 Gy) of high-LET radiations (80–300 keV/μm). In contrast, the amount of chromatin-bound Rad51 increased up to 15 Gy and decreased at 30 Gy after X-ray irradiation (data not shown).

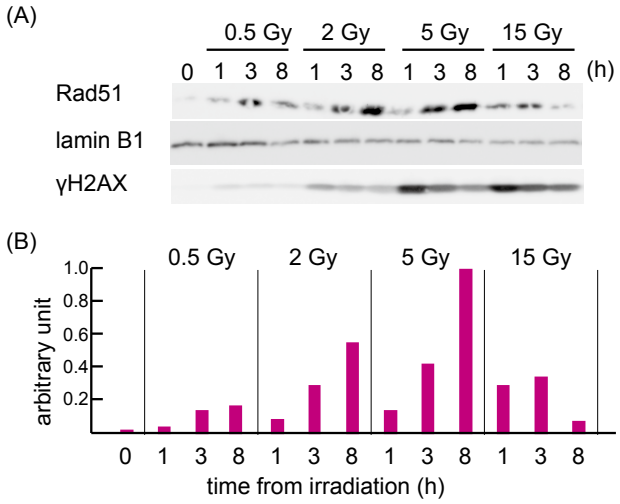

Fig. 1. Immunoblot analysis of chromatin-bound Rad51 after heavy-ion irradiation. (A) HeLa cells were irradiated with indicated doses of Ar ions, and 0.1% Triton-insoluble fractions (chromatin franctions) were prepared at indicated time points and subjected to immunoblot analysis. Phosphorylated histone H2AX (γH2AX) and lamin B1 were detected as an indicator of DSB and a loading control, respectively. (B) The relative intensity of Rad51 band was measured using luminoimage analyzer and normalized against the amount of lamin B1.

We previously reported that trichostatin A (TSA), a histone deacetylase inhibitor, enhanced radiosensitivity at low doses, whereas TSA suppressed it at high doses of heavy-ion irradiation by an unknown mechanism.[7] This finding also suggests that DNA damage response induced by heavy-ion irradiation depends on the dose. Currently, we are investigating the localization of several repair proteins involved in NHEJ and SSA after irradiation of different doses.

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References
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