Localization of Rad51 and phosphorylated DNA-PKsc after heavy-ion irradiation in mammalian cells

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Accelerated heavy-ion particles with high linear energy transfer (LET) induce complex clustered DNA damage, which is considered an obstacle to efficient repair and causes different biological effects compared with low-LET radiation such as X-ray. However, the biological effects of heavy-ion irradiation are not fully understood at the molecular level. To analyze the repair mechanism for DNA double-strand breaks (DSBs) caused by heavy ions, we have investigated cell sensitivity to heavy ions using the wild-type CHO-AA8 cells and two CHO mutant lines deficient in homologous recombination (HR)[1] or non-homologous end-joining (NHEJ)[2] in a previous study[3] and found that HR, but not NHEJ, is primarily involved in the repair pathway induced by high-LET ionizing radiation.

In this study, we investigated the localization of Rad51 and the phosphorylated form of the catalytic subunit of the DNA-dependent kinase (DNA-PKcs) in CHO cells after X-ray or heavy-ion irradiation with immunoflorescence staining. Rad51 is essential for HR and is involved in strand transfer during homologous pairing,[4] whereas DNA-PKcs is involved in NHEJ and phosphorylated upon DNA damage on threonin-2609.[5]

One hour after X-ray irradiation, hundreds of Rad51 or phosphorylated DNA-PKcs foci were observed in nuclei (Fig. 1A), although they were not colocalized with each other. The number of foci started to decrease 4 h after irradiation and fractions of Rad51 and phosphorylated DNA-PKcs foci were colocalized. A small fraction of DSBs was not repaired within 24 h (data not shown), and the foci remaining at 16 h after irradiation contained both Rad51 and phosphorylated DNA-PKcs, suggesting that irreparable DSBs recruite repair proteins of the NHEJ and HR pathways.

On the other hand, large fractions of Rad51 and phosphorylated DNA-PKcs foci were colocalized 1 h after iron-ion irradiation (LET = 1000 keV/μm) (Fig. 1B), suggesting that the majority of DSBs have different structures in nature. Although our previous results suggest that NHEJ is not involved in the repair pathway after heavy-ion irradiation,[3] we observed that phosphorylated DNA-PKcs was recruited to DSBs. These results suggest that repair proteins of both pathways are recruited to DSBs induced by heavy ions, and finally HR is selected for damage repair.

Currently, we are investigating how the cell cycle and LET affect the repair kinetics and colocalization of Rad51 and phosphorylated DNA-PKcs. We are also planning to examine the localization of 53BP1 and Rif1 after heavy-ion irradiation, which are involved in the selection of repair pathways for DSB repair.[6,7]

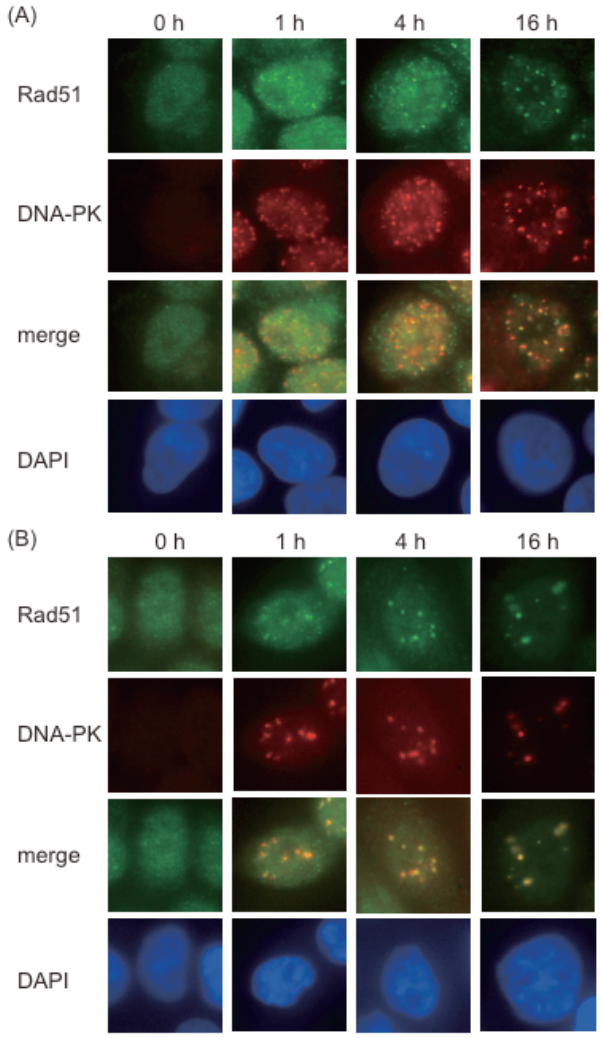

Fig. 1 Representative images of CHO nuclei (blue) with Rad51 (green) and phosphorylated DNA-PKsc (red) foci. The cells were irradiated with 5 Gy of X-ray (A) or iron ions (B) and fixed with 4% paraformaldehyde at indicated time points after irradiation. Foci formation of Rad51 and phosphorylated DNA-PKcs was detected by immunofluorescence staining.

References

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