Recruitment of Rad51 and phosphorylated DNA-PKcs after heavy-ion irradiation of human normal fibroblast

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DNA double-strand breaks (DSBs) caused by exposure to ionizing radiation are the most lethal type of damage because an accumulation of misrepaired or unrepaired DSBs can lead to a loss of genetic information and cell death. Accelerated heavy-ion particles with a high linear energy transfer (LET) induce complex clustered DNA damage including DSBs, which is considered to be an obstacle to efficient repair. DSBs are repaired primarily by non-homologous end joining (NHEJ) or homologous recombination (HR) in mammalian cells.[1] Our previous studies using the wild-type CHO cell and two CHO mutant lines deficient in HR or NHEJ suggest that HR is essential for survival after exposure to high-LET ionizing radiation.[2] However, several lines of evidence suggest that NHEJ is also involved in the repair of DSBs caused by high-LET ionizing radiation,[3,4] and the repair mechanism remains controversial in higher eukaryotes.

In this study, we investigated the foci formation of Rad51 and phosphorylated DNA-PKcs (catalytic subunit of DNA-dependent protein kinase), which are involved in HR and NHEJ, respectively (Fig. 1). We used human normal fibroblast NB1RGB cells since immortal cell lines often lose genetic stability or checkpoint control. The number of Rad51 foci and the percentage of Rad51foci-positive cells were maximized 3 h after X-ray irradiation (Figs. 1A, B). On the other hand, the number of Rad51 foci was almost maximized at 1 h after C (LET = 80 keV/μm) or Ar-ion (LET = 300 keV/μm) irradiation (Fig. 1A), suggesting that high LET radiation stimulates HR. In NB1RGB cells synchronized at a quiescent state (at the G0 phase) by serum starvation, the formation of Rad51 foci was not observed after X-ray, C-ion, or Ar-ion irradiation since HR is dependent on DNA replication. The number of Rad51 foci and the percentage of Rad51 positive cells gradually decreased as time proceeded after both X-ray and heavy-ion irradiation (Figs. 1A, B).

The number of phosphorylated DNA-PKcs foci in quiescent NB1RGB cells was twice that in logarithmically growing NB1RGB cells 1 h after X-ray irradiation (Fig. 1C), suggesting that NHEJ and HR work competitively. In contrast, the number of phosphorylated DNA-PKcs foci in quiescent cells was almost the same as that in logarithmically growing cells after C or Ar-ion irradiation. These results suggest that NHEJ does not recognize the fraction of DSBs caused by heavy-ion irradiation, which is consistent with the previous reports that HR is more relevant in the repair of complex DNA damage.[5,6] In addition, the number

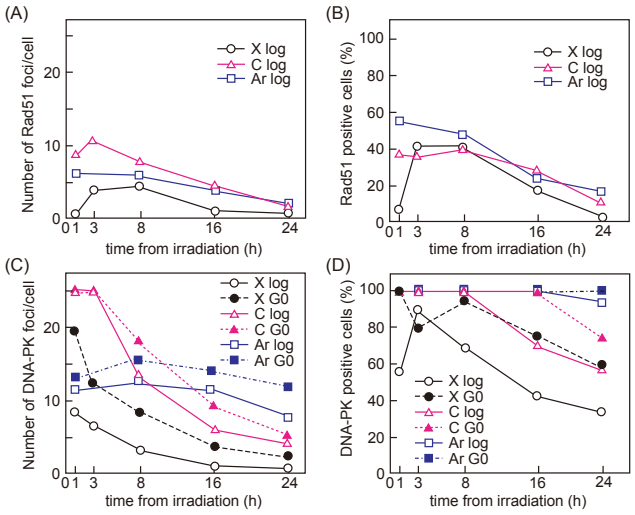

Fig. 1. Kinetics of the foci formation of Rad51 (A, B) and phosphorylated DNA-PKcs (C, D) in logarithmically growing NB1RGB cells (log) or synchronized cells at a quiescent state (G0). Cells were irradiated by 5 Gy of X-rays (circles), carbon-ions (triangles), and argon-ions (squares), and foci were detected by indirect immunofluorescent staining 1–24 h post irradiation.

of phosphorylated DNA-PKcs foci and the percentage of foci-positive cells decreased gradually as time proceeded after both X-ray and C-ion irradiation, whereas the number of phorphorylated DNA-PKcs foci persisted 24 h after Ar-ion irradiation in quiescent cells (Figs. 1C, D), suggesting that NHEJ does not efficiently repair DSBs after Ar-ion irradiation. We observed that 53BP1 and Rif1, which facilitate NHEJ, were co-localized with phosphorylated DNA-PKcs after the Ar-ion and X-ray irradiation (data not shown). Therefore, even though the components for the initial step of NHEJ were recruited to DSBs and DNA-PKcs was activated, NHEJ was impaired at a later step after Ar-ion irradiation.

References

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