Focus formation of Rad51 and phosphorylated DNA-PK 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. DNA double-strand breaks (DSB), the most dangerous DNA damage, are repaired primarily by non-homologous end joining (NHEJ), homologous recombination (HR), or microhomology mediated end joining 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 primarily involved in the repair pathway induced by high-LET ionizing radiation.[2,3] However, several studies suggest that NHEJ is also involved in DSB repair caused by high-LET ionizing radiation,[4,5] and the repair mechanism is still controversial in higher eukaryotes.

In this study, we investigated the foci formation of Rad51 and phosphorylated DNA-PK, which are involved in HR and NHEJ, respectively (Fig. 1). In human normal fibroblast NB1RGB cells synchronized at the G0 phase by serum starvation, the formation of Rad51 foci was not observed after X-ray or Ar-ion irradiation since HR is dependent on the S phase. The number of Rad51 foci reached maximum 8 h after X-ray irradiation and decreased gradually thereafter in both HeLa cells and logarithmically growing NB1RGB cells. On the other hand, the number of Rad51 foci increased immediately after Ar-ion irradiation (LET = 300 keV/μm), suggesting that the high LET radiation stimulates HR. The number of Rad51-positive cells in the population of HeLa and logarithmically growing NB1RGB cells also reached maximum at 8 h after X-ray irradiation. In contrast, the number of Rad51-positive cells in the population of HeLa cells increased with time after Ar-ion irradiation, whereas that in the population of logarithmically growing NB1RGB cells decreased as time proceeded because DNA damage caused by Ar-ion irradiation induced prolonged cell cycle arrest at the G2 and G1 phase in HeLa and NB1RGB cells, respectively.

The number of phosphorylated DNA-PK foci in quiescent NB1RGB cells was twice that in logarithmically growing NB1RGB cells 1 h after X-ray irradiation, suggesting that NHEJ and HR work competitively. In contrast, the number of phosphorylated DNA-PK foci in quiescent cells was slightly higher than that in logarithmically growing cells after Ar-ion irradiation. These results suggest that HR works mainly after Ar-ion irradiation, which is consistent with our previous report[5]. All the irradiated HeLa and quiescent NB1RGB cells had the DNA-PK foci 1 h after X-ray irradiation, whereas only 57% of logarithmically growing NB1RGB cells had the DNA-PK foci 1 h after irradiation, suggesting that the foci formation of phosphorylated DNA-PK occurs only in the G1-S phase in NB1RGB cells after X-ray irradiation. In contrast, the foci of phosphorylated DNA-PK were observed in all irradiated cells 1 h after Ar-ion irradiation, suggesting that the foci formation of phosphorylated DNA-PK occurs even in the G2 phase after Ar-ion irradiation. These results also suggest that the regulation of formation of DNA-PK foci was different between X-ray and Ar-ion irradiation. Now we are investigating the localization of the other repair proteins involved in the selection of repair pathways for DSB repair.

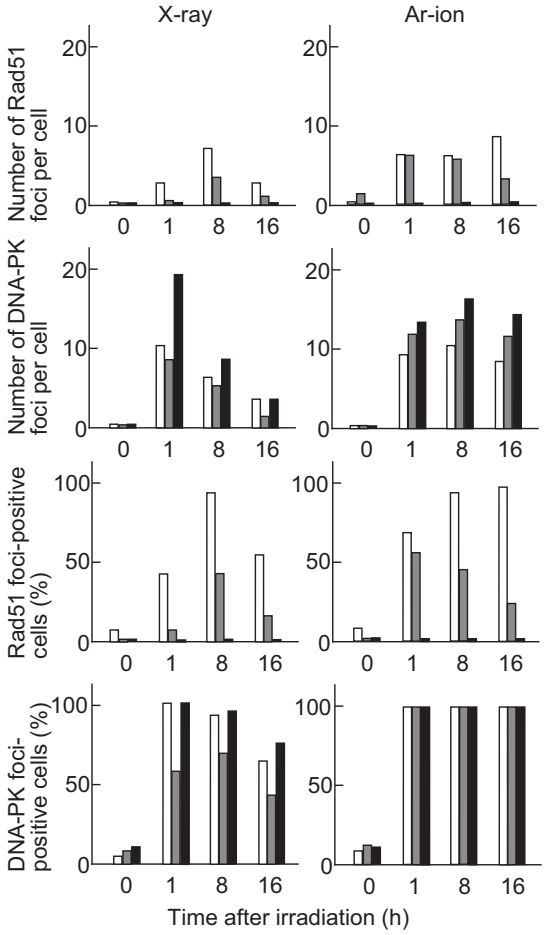

Fig. 1 Kinetics of the foci formation of Rad51 and phosphorylated DNA-PK in HeLa cells (open box), logarithmically growing NB1RGB cells (gray box), and synchronized NB1RGB cells at the G0 phase (closed box). The foci were detected by indirect immunofluorescent staining 1-16 h post irradiation.

References

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