The inhibitor of DNA-PK suppressed DNA repair after heavy-ion irradiation in quiescent mammalian cells

M. Izumi*1 and T. Abe*1

Accelerated heavy-ion particles with high linear energy transfer (LET) induce complex clustered DNA damage, which is an obstacle to efficient repair. DNA double-strand breaks (DSBs) are most lethal damage among them and are repaired primarily by non-homologous end joining (NHEJ) or homologous recombination (HR) in mammalian cells, whereas alternative NHEJ (alt-NHEJ) and/or single strand annealing (SSA) work only when both NHEJ and HR are impaired.

Several published studies using the Chinese hamster ovary (CHO) cells and two CHO mutant lines deficient in HR or NHEJ suggest that NHEJ does not efficiently repair DNA damages and that HR is essential for survival after heavy-ion irradiation.[1,2] On the other hand, a study using inhibitors against NHEJ and HR in human lung cancer cell line suggests that NHEJ is a major DNA repair pathway after heavy-ion irradiation.[3] Therefore, the DNA repair mechanism is still controversial in higher eukaryotes.

In this study, we investigated the repair mechanism in quiescent mammalian cells, where HR does not work. To estimate the secondary carcinogenesis in radiation therapy, it is important to know whether DNA damages are repaired by NHEJ or highly mutagenic alt-NHEJ and/or SSA, because the majority of the cells in the body exist in a quiescent state. First, we investigated the foci formation of phosphorylated histone H2AX, which reflects the presence of DSBs after irradiation (Fig. 1). The number of histone H2AX foci reached maximum immediately after X-ray or carbon ion irradiation and decreased similarly as time proceeded in both logarithmically growing cells and quiescent cells. These results suggest that NHEJ is a major repair pathway in both logarithmically growing cells and quiescent cells since HR does not work in quiescent cells and requires a much longer time ($>$ 8 h) than NHEJ.

To confirm that NHEJ is a major pathway in quiescent cells, we investigated the effect of NU7441, a potent specific inhibitor of DNA-PK, which is involved in NHEJ (Fig. 2). NU7441 increased the number of phosphorylated histone H2AX 16 h after carbon ion irradiation, suggesting that NHEJ is the dominant DNA repair pathway. It is also suggested that alt-NHEJ or SSA does not work efficiently in quiescent cells.

In the previous report, we have shown that the cell survival of CHO cells is dependent on HR[4] as several groups have already reported.[1,2] HR may play a major role in DNA repair in CHO cells because CHO cells are not arrested in the G1 phase and accumulate in the S-G2 phase after irradiation due to the lack of p53

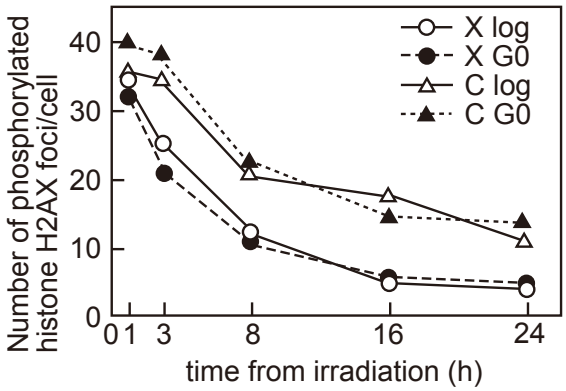

Fig. 1. The number of phosphorylated histone H2AX foci after irradiation. NB1RGB cells were synchronized in the quiescent state by serum starvation for 48 h. Cells in the proliferating state (○ △) or quiescent state (●) were irradiated with 5 Gy of X-ray or carbon ions (LET = 80 keV/μm), and the phosphorylated histone H2AX was detected by immunostaining 1–24 h post irradiation.

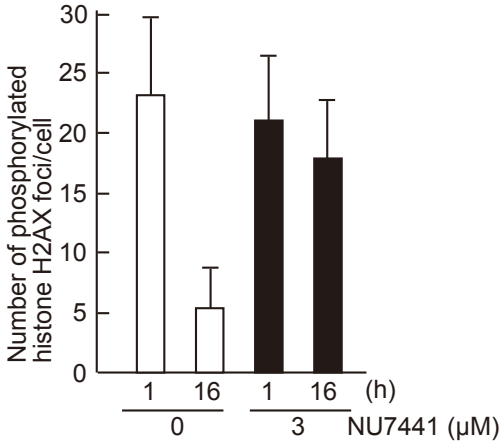

Fig. 2. The effect of NU7441 on DNA repair. The quiescent NB1RGB cells were treated with 3 μM NU7441 and irradiated with 2 Gy of carbon ions, and the phosphorylated histone H2AX was detected 16 h post irradiation.

(data not shown). On the contrary, the majority of logarithmically growing NB1RGB cells (60–70%) are in the G1 phase and stay in the G1 phase until DSB repair is completed. Therefore, NHEJ may play a major role in DNA repair in NB1RGB cells. Currently, we are examining the effect of drugs on several cell lines to investigate whether the genetic background can explain the selection of repair pathways.

References

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*1 RIKEN Nishina Center