Heavy-ion irradiation enhanced the post-transcriptional modification of CtIP

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Among DNA damages caused by ionizing radiation, DNA double strand breaks (DSBs) are the most lethal as misrepaired or unrepaired DSBs can cause a loss of genetic information, canceration, 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). Accelerated heavy-ion particles with high linear energy transfer (LET) induce complex clustered DNA damage including DSBs, single strand breaks, and base damages within one or two helical turns of DNA. The clustered DNA damage is considered as an obstacle to efficient repair and affects the pathway choice for DSB repair.

After exposure to low-LET radiation, NHEJ is the dominant repair pathway throughout the cell cycle, whereas HR works only in the late S/G2 phase. Alt-NHEJ and SSA are considered to be functional only when both the NHEJ and HR are impaired.[1)] 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 suggests that NHEJ is the major repair pathway after 2 Gy of heavy-ion irradiation.[2)] In addition, we have shown that HR is favored after heavy-ion irradiation in G2-phase, although HR repairs DSBs less efficiently after heavy-ion irradiation than after X-ray irradiation.[3)] Moreover, recruitment of Rad51 (HR factor) to DSB is suppressed by high dose (>15 Gy) heavy-ion irradiation,[4)] suggesting that the pathway choice is dependent on the cell cycle, LET, and dose.

In this study, we examined the modification of CTBP-interacting protein (CtIP) after heavy-ion irradiation. CtIP is involved in end-resection of DSBs for generating 3′-single strand DNA, which promotes HR, SSA, and alt-NHEJ.[5)] CtIP is phosphorylated by active ATM as well as cyclin-dependent kinase in S/G2 phase. Phosphorylation of CtIP facilitates its interaction with BRCA1 and Mre11/Rad50/Nbs1 nuclease complex. Recent studies suggest that the balance between 53BP1-Rif1 and CtIP-BRCA1 controls the pathway choice. Thus, the choice between NHEJ and HR/alt-NHEJ/SSA is determined by which among end-joining or end resection occurs first.[6)]

Exponentially growing HeLa cells were irradiated with argon ions (LET = 300 keV/μm) of different doses (2–30 Gy), and chromatin fractions were obtained and subjected to an immunoblot analysis (Fig. 1). In unirradiated cell extracts (at 0 hour), both phosphorylated and non-phosphorylated forms of CtIP were detected. The level of phosphorylated forms increased after both 15 and 30 Gy of X-ray irradiation. Argon-ion irradiation induced phosphorylation at the lower dose (2–5 Gy), suggesting that the end-resection is enhanced after heavy-ion irradiation. In addition, we found a more retarded form of CtIP after high dose (>15 Gy) of argon-ion irradiation. The retarded form may be hyperphosphorylated or modified by small proteins such as ubiquitin or SUMO. Moreover, we observed the same form after the carbon-ion (LET = 80 keV/μm) irradiation (data not shown).

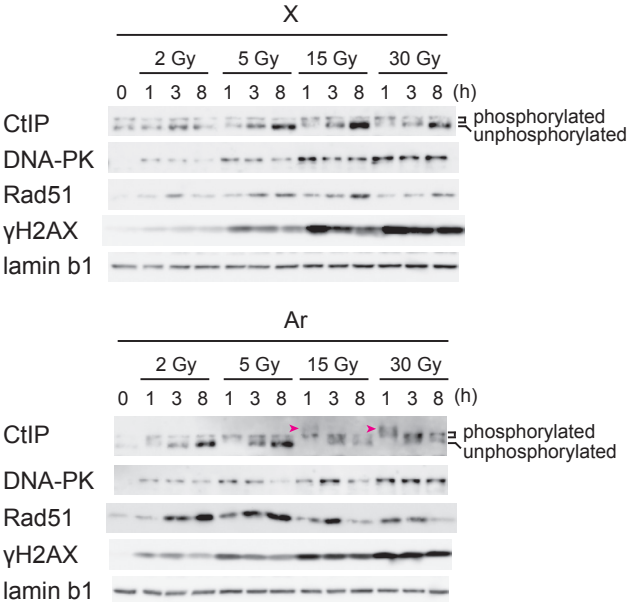

Fig. 1. Immunoblot analysis of chromatin-bound repair proteins after irradiation. HeLa cells were irradiated with indicated doses of X ray or Ar ions. Triton-insoluble fractions (chromatin fractions) were prepared at indicated time points following irradiation and subjected to immunoblot. CtIP, phosphorylated DNA-PK, Rad51, and phosphorylated histone H2AX (γH2AX) were detected. Arrowheads indicate the retarded modified forms, which were absent in X-ray irradiated extracts. Lamin B1 was detected as a loading control.

We also detected phosphorylated DNA-PK (NHEJ factor) and Rad51 (Fig. 1). The amount of phosphorylated DNA-PK was dependent on the dose, whereas that of Rad51 increased up to 5 Gy after argon-irradiation and decreased at 15 Gy irradiation as previously reported.[4)] These results suggest that CtIP can promote alt-NHEJ and/or SSA at high doses (>15 Gy).

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References
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