Low-dose high-LET heavy ion-induced bystander signaling (IV)

M. Tomita,[*1,*2] T. Tsukada,[*2] and M. Izumi[*2]

Radiation-induced bystander response (RIBR) is a cellular response induced in non-irradiated cells that received bystander signals from directly irradiated cells within an irradiated cell population.[1)] RIBR induced by low doses of high-LET radiation is an important issue for the health of astronauts and in hadrontherapy. Here, we investigated the underlying molecular mechanisms and biological implications of RIBR induced by such low doses of high-LET radiation.

We found that normal human fibroblasts cultured confluent, which were harvested 16–24 h after exposure to high-LET (1000 keV/μm) iron (Fe) ions, showed the cell killing effect at low doses ($\leq$ 0.2 Gy) higher than that estimated by a linear extrapolation from high doses. This enhanced cell killing effect could not be observed in the cells harvested immediately after irradiation.[2)] At 0.1 Gy, the average number of Fe-ion traversals per cell nucleus was 0.11; however, the surviving fraction was 0.84.[3)] These results suggested that the enhanced cell killing effect at low doses was at least partly caused by the induction of bystander responses. In addition, we established an optimal system to assess the low doses of high-LET radiation-induced bystander cell killing, and reported that gap-junction intercellular communication (GJIC), cyclooxygenase-2 (COX-2), and nitric oxide (NO) were involved in its signal transfer.[3)]

In our previous study using high-LET heavy-ion microbeam and broadbeam,[4)] we showed that DNA double-strand breaks (DSBs) and reproductive cell death were induced by NO-mediated bystander response in normal human fibroblasts. In addition, the activation of NF-κB, Akt, and COX-2 by bystander signaling depended on incubation time after irradiation and presence of NO. In this study, we investigated phosphorylation and accumulation of these bystander signaling related molecules in the cells irradiated with low doses of high-LET radiation.

Figure 1 shows phosphorylation and accumulation of bystander signaling related molecules in normal human fibroblasts, WI-38, irradiated with 0.1 Gy of 90 MeV/u Fe ions (1000 keV/μm). WI-38 cells were cultured on 25 cm^2 plastic flasks for 1 week to form confluent monolayers and were pretreated with or without a scavenger of NO, c-PTIO, (20 μM) 2 h before irradiation with 0.1 Gy of Fe ions. Cells were harvested 3 and 6 h after irradiation followed by immunoblotting. Phosphorylation of Akt at Ser473 and NF-κB p65 at Ser536 and accumulation of COX-2 were observed in the cell 3 h after irradiation and were efficiently inhibited by pretreatment with c-PTIO. Phosphorylated histone H2AX at Ser139 is widely used as a surrogate marker of DSBs. Phosphorylated histone H2AX was observed at 3 and 6 h after irradiation. Prolonged phosphorylation of H2AX at 6 h after irradiation was inhibited by c-PTIO, although phosphorylation at 3 h was not suppressed. NO-mediated prolonged phosphorylation of H2AX also indicated the induction of bystander responses. These results suggest that NF-κB/COX-2/prostaglandin E2 and NF-κB/iNOS/NO pathways[1,5)] are activated in the cells irradiated with low doses of high-LET radiation.

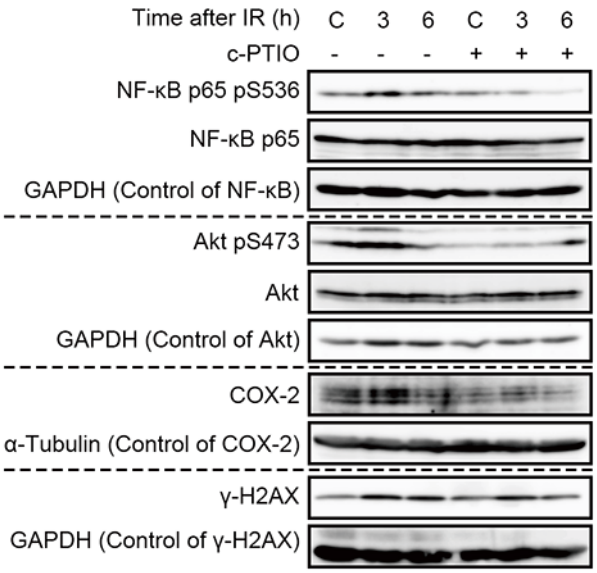

Fig. 1. Phosphorylation and accumulation of bystander signaling related molecules. Normal human fibroblasts, WI-38, were pretreated with or without c-PTIO (20 μM) 2 h before irradiation with 0.1 Gy of 90 MeV/u Fe ions (1000 keV/μm). Cells were harvested 3 and 6 h after irradiation followed by immunoblotting.

References

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*1 Radiation Safety Research Center, Central Research Institute of Electric Power Industry
*2 RIKEN Nishina Center