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

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 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) are involved in its signal transfer.[2)] Figure 1 shows the results obtained in these experiments. Normal human fibroblasts, WI-38, were irradiated with 0.1 Gy of 90 MeV/u Fe ions (1000 keV/μm). Cells were harvested 16–24 h after irradiation and plated on cell culture dishes to allow colony formation. The surviving fraction decreased to 0.84 ± 0.02 by the bystander cell killing effects (Fig.1, control). Lindane (Lin) and NS-398 (inhibitors of GJIC and COX-2, respectively) were dissolved in DMSO (a scavenger of reactive oxygen species). c-PTIO is a scavenger of NO. DMSO (0.1%), Lin (50 μM), c-PTIO (20 μM), or NS-398 (50 μM) was added to the medium 2 h before irradiation.[3)] DMSO did not significantly suppress bystander cell killing. In contrast, Lin, NS-398, and c-PTIO significantly ($P < 0.05$) suppressed cell death to similar levels. Cells pretreated with both c-PTIO and Lin did not survive more than those pretreated with Lin or c-PTIO alone. These results support our previous suggestion that GJIC is involved in bystander signaling and the cell culture medium induces the bystander cell killing effect in a coordinated manner.[2)]

The fluence (number of ion tracks/cm^2) was calculated as the dose (Gy)/LET (keV/μm)/(1.6×10^{-9}). The area of the cell nucleus was 188 μm^2. The average number of ion tracks per cell nucleus was calculated as 0.11 when cells were irradiated with 0.1 Gy of Fe ions (1000 keV/μm).[4)] To confirm this result, we measured the number of ion hits per cell nucleus using a plastic ion-track detector, CR-39. The CR-39 film was irradiated with 0.1 Gy of Fe ions and then cells were cultured on the film. After cells were fixed and stained with the DAPI solution, the opposite surface of the film was etched with an alkaline-ethanol solution.[5)] Figure 2 shows the images of cell nuclei and the etch pits of the CR-39 film. The number of ion hits per cell nucleus was 0.11 ± 0.01 (n=5), which corresponded with the calculated results. Therefore, the cell surviving fraction at a dose of 0.1 Gy was lower than the number of Fe-ions hit on the cell nucleus, suggesting that cell death was induced in non-hit cells by RIBR.

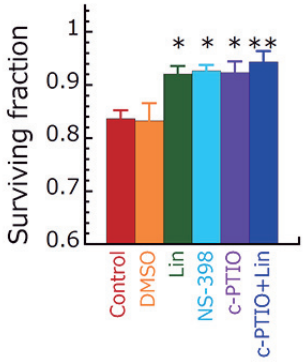

Fig. 1. Effect of inhibitors or scavengers on cell survival. WI-38 cells pretreated with inhibitors or scavengers were irradiated with 0.1 Gy of Fe ions (1000 keV/μm). Error bars represent the standard errors of the means (SEMs) (n=4–6).*$P < 0.05$ and **$P < 0.01$, for comparison with control and drug-treated cultures.

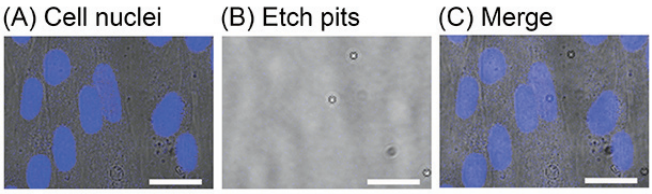

Fig. 2. Microscopic images of WI-38 cell nuclei and etch pits of the CR-39 irradiated with 0.1 Gy of Fe ions (1000 keV/μm). Panel A shows the fluorescence image of WI-38 cell nuclei stained with DAPI. Panel B shows the etch pits of the CR-39 film. Panel C shows the overlay of A and B. Scale bar is 20 μm

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

References

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