3 - 77 Induction of Micronuclei in Bystander Cells after X-ray and Carbon Ion Irradiation

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Since the first report by Nagasawa et al.[1] in 1992, which demonstrates evidences that non-irradiated bystander cells also showed damage of sister chromatid exchange in samples irradiated with low-dose α particles, radiation induced bystander effect (RIBE) has been studied for more than decades and many publications showed that RIBEs were found after treatment of both low and high-LET radiations. However, there are also some reports showing contrary facts that no obvious bystander effects can be found, for example Fournier et al.[2] reported that they observed no early cytogenetic damage in the bystander cells after heavy ion microbeam irradiation. RIBE is of great interest in radiotherapy using X-ray radiation or high-LET heavy ions because of RIBE-related cell killing and carcinogenesis in neighboring normal cells[3]. In this work, we investigated the damage in bystander cells exposed to medium from cells irradiated with X-ray and carbon beam.

We first irradiated the human bronchial epithelial cells HBEC-3KT with 5 Gy X-rays (80 kV) and 2 Gy carbon ions (160 MeV/u, LET $\sim$ 20 keV/μm) respectively, which were cultured in the 12-well culture plate with an area of 4 cm^2(about 10^5 cells). Then the HBEC-3KT cells were co-cultured with the primary normal human lung fibroblasts MRC-5 cells for 30 min. Micronuclei (MN) formation was examined in the MRC-5 cells. The results show that significant bystander effect of MN was found in MRC-5 cells induced by both X-ray and carbon ions, in average 10 and 17 MNs were induced in $\sim$1 000 cells by X-ray and carbon ions, respectively (Fig. 1).

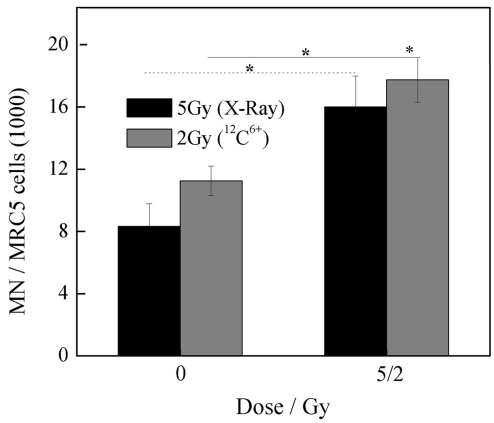

Fig. 1 (color online) The induction of MNs in the MRC-5 cells. At least 1 000 cells were scored for each sample and the data were pooled from 3$\sim$4 independent experiments. The data show the mean standard error.

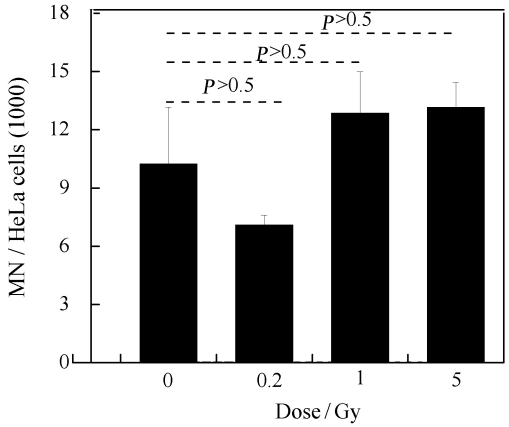

Fig. 2 (color online) The induction of MNs in the HeLa cells. More than 1 500 cells were counted in each sample and the data shows the mean and standard deviation of three samples.

We then investigated bystander effect in HeLa cells cultured in medium transferred from HeLa cells irradiated by carbon microbeam (80 MeV/u, LET $\sim$ 0.25 MeV/(mg/cm^2), in which a region of 380 μm$\times$500 μm (about 30$\sim$50 cells) in the ϕ35 mm Petri dish was micro-irradiated with a dose of 0, 0.2, 1, 5 Gy. The irradiated cells were cultured for 17 h and then their medium was transferred to the recipient cell dish. After another 17 h, the recipient cells were fixed and MN was counted. The results showed that there was about 10 MNs for every 1 000 cells and no significant difference was found (Fig. 2).

In this study, the micronuclei assay were performed with 0.1% acridine orange staining and no cell cycle blocking agent was used, such as cytochalasin-B which can enhance the number of binucleated cell population. This RIBE difference in the micronuclei formation may result from the cell type, irradiation protocol or the culture method.

References

[1] H. Nagasawa, J. B. Little, Cancer. Res, 52(1992)6394.

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[3] C. Mothersill, C. Seymour, Radiat. Res, 155(2001)759.