RELATION BETWEEN RADIO-ADAPTIVE RESPONSE AND CELL TO CELL COMMUNICATION

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INTRODUCTION

Ionizing radiation has been considered to cause severe damages to DNA and do harm to cells in proportion to the dose, however low it might be. In 1984, Wolff et al. showed that human peripheral lymphocytes adapted to the low-dose radiation from ^{3}H-TdR added in culture medium and became resistant to the subsequent irradiation with high-doses of X-rays (1). This response, which is called radio-adaptive response, is also induced by X-rays and gamma-rays in human lymphocytes (2) and Chinese hamster V79 cells (3). However, the mechanisms of and conditions for adaptive responses to radiation have not been clarified. With an objective of clarifying the conditions for adaptive responses of cells to radiation, we examined how the cell to cell communication is involved in the adaptive responses. We irradiated normal human embryo-derived (HE) cells and cancer cells (HeLa) in culture at high density with low-dose X-ray and examined their radio-adaptive responses by measuring the changes in sensitivity to subsequent high-dose X-ray irradiation using the Trypan Blue dye-exclusion test method. We also conducted experiments to examine the effects of Ca^{2+} ions and Phorbol 12-Myristate 13-Acetate (TPA) which are supposed to be involved in cell to cell communication.

MATERIALS AND METHODS

(1) Dose-dependency of Preliminary Irradiation Effect

Four hours after the start of culture of HE cells, the cells were irradiated with low doses of X-ray ranging from 0 to 20 cGy. Similarly, HeLa cells were irradiated with X-ray ranging from 0 to 40 cGy. The cells were cultured for another four hours, then subjected to high-dose irradiation of 200 cGy. After high-dose irradiation, the cells were cultured for five days before the number of viable cells was counted. The relative growth ratios of the respective groups were determined. The control groups received mock treatments for both the low-dose irradiation and high-dose irradiation.

(2) Effects of Calcium Ions and TPA

Four hours after the start of the culture of the HE cells, the regular culture medium was replaced by Ca^{2+}-containing Hank's Balanced Salt Solution (HBSS) or Ca^{2+}-free HBSS. Then each group received irradiation of 13 cGy or mock treatment. Ten minutes after the irradiation

or the mock treatment, the HBSS were replaced by the regular medium. Then the cells were cultured for four hours before receiving a high-dose irradiation of 200 cGy. TPA was added to the culture medium at a concentration of 100 ng/ml at 3 hours and 40 minutes after the start of culture of HE cells, then the cells were irradiated with 13 cGy 20 minutes after the addition of TPA. The culture medium was removed 20 minutes after irradiation at 13 cGy, the cell sheet was rinsed 3 times with PBS, and the regular culture medium was poured to the culture flask. The cells were cultured for 4 additional hours, and then irradiated with 200 cGy. After that, the cells were cultured for five days, and the numbers of viable cells were counted to determine the relative growth ratios of the cultures. The control groups were treated in the same manner as the irradiated groups except that both the low-dose irradiation and the high-dose irradiation were mock treatments.

RESULTS AND DISCUSSION

(1) Dose-dependency of Preliminary Irradiation Effect

The results are shown in Fig. 1. When a high-dose irradiation of 200 cGy was given to HE cells, the growth ratio of the living cells decreased to 37 % of that of the control. When a preliminary irradiation of 10 to 20 cGy was given to HE cells four hours before the irradiation of 200 cGy, the relative growth ratio significantly increased to 45-53 %; and a peak was reached at a preliminary irradiation dose of 13 cGy. In HeLa cells, however, preliminary irradiation by the entire dose range from 0 to 40 cGy did not affect the relative growth ratio after 200 cGy irradiation.

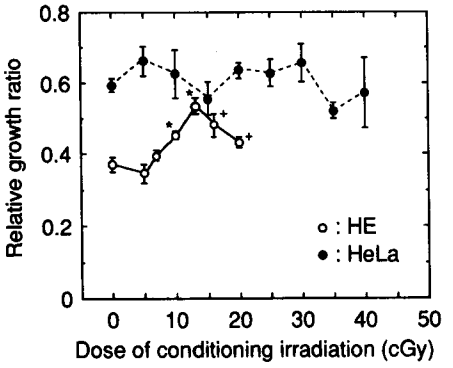

Fig. 1 Changes in relative growth ratio of pre-irradiated human embryonic cells and HeLa cells after receiving the challenging irradiation of 200 cGy, as a function of pre-irradiated conditioning dose. Relative growth ratio = growth ratio of irradiated cells / growth ratio of sham-irradiated control. Each point is the mean ± SD of six independent determinations. $^{*}p<0.01$, $^{+}p<0.05$ by paired t-test.

(2) Effects of Calcium Ions and TPA

The results are shown in Fig. 2. When the HE cells were given an irradiation of 200 cGy without any preliminary irradiation, the growth ratios of the living cells decreased to 31-37 % of those of the control groups irrespective of the presence or absence of Ca^{2+} in the HBSS

which replaced the regular medium. When an irradiation of 13 cGy was given to the HE cells in the Ca^{2+}-containing HBSS four hours before the 200 cGy irradiation, the relative growth ratio of the living cells increased significantly to 53 %. However, when the HE cells were given an irradiation of 13 cGy in the Ca^{2+}-free HBSS, the relative growth ratio of the living cells was about 32 %.

When TPA was added to the culture medium at 100 ng/ml during preliminary irradiation, the relative growth ratio of HE cells significantly decreased to 42%.

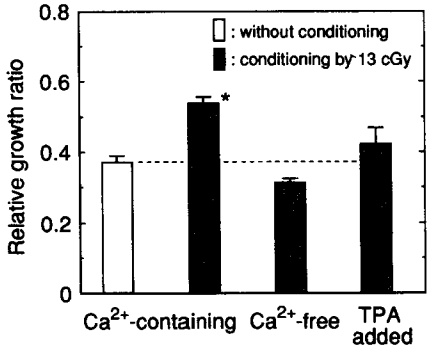

Fig. 2 Changes in relative growth ratio of pre-irradiated human embryonic cells after receiving the challenging irradiation of 200 cGy by the presence or absence of calcium ions and TPA in the medium during the conditioning irradiation. Each point is the mean ± SD of six independent determinations. *$p<0.05$ by paired t-test.

In the present study, to examine how cell to cell communication is involved in the adaptive responses of cells to radiation, we irradiated normal cells (HE) and cancer cells (HeLa) with low-dose X-ray and examined changes in their sensitivity to subsequent high-dose X-ray irradiation. As a result, the preliminary irradiation of the HE cells by low-dose X-ray moderated the decrease in the growth ratio of the cells due to the subsequent 200 cGy irradiation, and increased the radioresistance. This adaptive response was not observed in HeLa cells. When the HE cells were suspended in a Ca^{2+} ion-free medium or TPA added medium while receiving the conditioning irradiation, the adaptive response was not observed. The above-mentioned findings suggest a good possibility that low-dose irradiation enhances the radio-resistance of normal cells. The results also show that there is an optimum dose range for inducing the radio-adaptive response. This radio-adaptive response seems to involve cell to cell communication maintained in the normal cells (4).

REFERENCES

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