Participation of Intercellular Communication and Intracellular Signal Transduction in the Radio-adaptive Response of Human Fibroblastic Cells

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Abstract

To investigate the radio-adaptive response of normal cells to low-dose radiation, we irradiated human embryonic cells with low-dose X-rays and examined the changes in sensitivity to subsequent high-dose X-irradiation. When the cells were irradiated by 200 cGy, the growth ratio of the viable cells five days after the irradiation decreased to 37 % of that of the cells which received no X-irradiation. When the cells received a conditioning irradiation of 10 to 20 cGy four hours before the irradiation of 200 cGy, the growth ratio increased significantly to 45-53 %, and a peak was reached at a conditioning dose of 13 cGy. Cells blocked off intercellular communication either in Ca^{2+} ion-free medium or in TPA added medium during the conditioning irradiation of 13 cGy did not show the improvement of growth ratio. Addition of H-7, as an inhibitor of PKC, to the medium during the conditioning irradiation inhibited the induction of the radio-adaptive response. However, addition of either inhibitor of A kinase, H-89, or inhibitor of G kinase, H-8, failed to inhibit the induction of the radio-adaptive response.

These results suggest that: (1) normal cells show an adaptive response to low-dose radiation, (2) intercellular communication may play a role in radio-adaptive responses, (3) the transduction of the signal induced in cells by low-dose X-irradiation via protein kinase C was involved in radio-adaptive responses, not via A kinase nor G kinase.

Introduction

In the past, radiation was considered to cause damages to DNA and do harm to cells in proportion to the dose, however low it might be. In 1984, Wolff et al. discovered that human lymphocytes which received ^{3}H-TdR treatment showed an adaptive response to radiation [1]. Radio-adaptive responses are also induced by X-rays and gamma rays in human lymphocytes [2], Chinese hamster V79 cells [3] and normal human embryo-derived fibroblastic (HE) cells [4]. However, the mechanisms of adaptive responses to radiation and the conditions for induction of it have not been clarified. Therefore, we examined how the intercellular communication and intracellular signal transduction are involved in radio-adaptive responses.

We irradiated HE cells in culture with low-dose X-rays and examined their radio-adaptive responses by measuring the changes in sensitivity to subsequent high-dose X-ray irradiation. To examine the effects of Ca^{2+} ions and TPA which are supposed to be involved in intercellular communication, we also conducted experiments in which Ca^{2+} ions were eliminated from the culture medium or TPA was added to the medium during the low-dose conditioning irradiation. We also inhibited proteins that play major roles in various pathways of intracellular signal transduction when radio-adaptive responses of HE cells were induced by conditioning irradiation. We evaluated the survival rates to investigate the pathway of intracellular signal transduction thought to be involved in the induction of radio-adaptive response.

Materials and Methods

(1) Dose-dependency of Conditioning Irradiation Effect

Four hours after the start of culture of HE cells, the cells were irradiated for conditioning with X-ray ranging from 0 to 20 cGy. The cells were cultured for another 4 hours, then subjected to challenging irradiation of 200 cGy. After challenging irradiation, the cells were cultured for 5 days before the number of viable cells was counted by the trypan blue dye-exclusion test method. The control groups received mock treatments for both the conditioning and challenging irradiation. The growth ratios of the respective groups to controls were determined.

(2) Effects of Calcium Ions and TPA

Four hours after the start of culture of HE cells, the regular medium was discarded and replaced by Ca^{2+}-containing Hank's Balanced Salt Solution (HBSS) or Ca^{2+}-free HBSS. Then each group received conditioning dose of 13 cGy or mock treatment. Ten minutes after the conditioning or the mock treatment, the HBSS were replaced by the regular medium again. Then the cells were cultured for 4 hours before receiving the challenging dose of 200 cGy. TPA was added to the medium at a concentration of 100 ng/ml at 3 hours and 40 minutes after the start of culture, and the cells were then irradiated with conditioning dose of 13 cGy 20 minutes after the addition of TPA. The medium was discarded 20 minutes after conditioning irradiation, the cell sheet was rinsed 3 times with PBS, and the fresh regular medium was poured to the culture flask. The cells were cultured for 4 additional hours, and then irradiated with challenging dose of 200 cGy. After that, the cells were cultured for 5 days, and the numbers of viable cells were counted to determine the growth ratios of the cultures. The control groups were treated in the same manner as the irradiated groups except that both the conditioning irradiation and the challenging irradiation were mock treatments.

(3) Effects of Inhibitors on Intracellular Signal Transduction

Various inhibitors of intracellular signal transduction were added to the culture medium at 3 h and 50 min after the start of culture of HE cells. At 10 min after addition of an inhibitor, the cells were irradiated with conditioning dose of 13 cGy, and the medium containing the inhibitor was then discarded 10 min later. After that, the cell surface was rinsed twice with PBS, and fresh

regular medium was poured into the flask. At 4 hours after conditioning irradiation, the cells were irradiated again with challenging dose of 200 cGy and cultured for another 6 days. Then, viable cells were counted. The intracellular signal transduction inhibitors used were H-89 as an inhibitor of cAMP-dependent protein kinase (A kinase), H-7 as an inhibitor of protein kinase C (PKC), and H-8 as an inhibitor of cGMP-dependent protein kinase (G kinase). In the control group, only DMSO, in which these inhibitors were dissolved, was added.

Results

(1) Dose-dependency of Conditioning Irradiation Effect

The results are shown in Fig. 1. The growth ratio of the viable cells after challenging irradiation of 200 cGy decreased to 37 % of that of the controls. While growth ratio of the cells exposed to 10 to 20 cGy four hours before the challenging irradiation significantly increased to 45-53 %; and a peak was reached at a conditioning irradiation dose of 13 cGy.

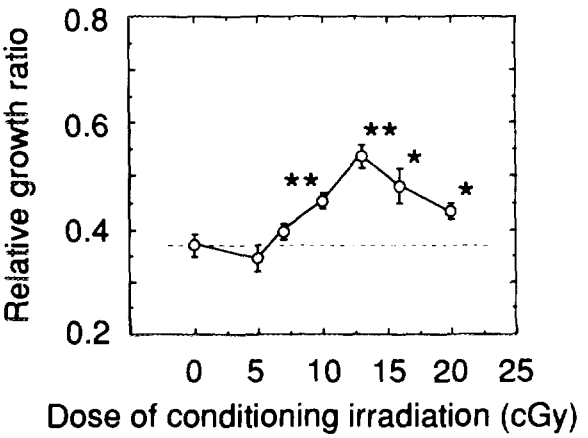

Fig. 1 Changes in growth ratio of the cells after receiving the challenging irradiation of 200 cGy, as a function of pre-irradiated conditioning dose. Each point is the mean ± SD of six independent determinations. **p<0.01, *p<0.05 by paired t-test.

(2) Effects of Calcium Ions and TPA

When HE cells were exposed to a challenging irradiation of 200 cGy with mock conditioning irradiation in HBSS with or without Ca^{2+}, the growth ratio of the viable cells decreased to 31-37 % of the control groups. When a conditioning irradiation of 13 cGy was given to HE cells in the Ca^{2+}-containing HBSS four hours before the challenging irradiation, the growth ratio of the viable cells increased significantly to 53 %. However, when HE cells were given a conndtioning irradiation in the Ca^{2+}-free HBSS, the growth ratio of the viable cells was about 32 %. When TPA was added to the culture medium at 100 ng/ml during the conditioning irradiation, the growth ratio of the viable cells significantly decreased to 42%.

(3) Effects of Inhibitors on Intracellular Signal Transduction

When H-89 or H-8 was added to the medium for only 20 min from 10 min before to 10 min after conditioning irradiation, no significant difference in number of the viable cells was observed. Viable cells significantly decreased in number by adding H-7, as compared with the control group.

Discussion

To examine the role of intercellular communication in the adaptive responses of cells to radiation, we irradiated normal HE cells with low-dose X-ray and examined changes in their

sensitivity to subsequent high-dose X-ray irradiation. As a result, the conditioning irradiation of 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 observed when the conditioning irradiation dose was in a range from 10 to 20 cGy. When conditioning irradiation was given to the cells in a Ca^{2+}-free medium, no radio-adaptive response was observed. Ca^{2+} ions are considered to be involved in intercellular communication. From the results of the experiment, in which HE cells in the culture medium containing TPA as an inhibitor on intercellular communication were irradiated at 13 cGy, it was confirmed that intercellular communication is involved in the radio-adaptive response. Therefore, it is possible to assume that intercellular communication is involved in the response of irradiated cells [5].

When H-7 was added to the medium before conditioning irradiation, the number of viable cells at 6 days after challenging irradiation significantly decreased, as compared with that of the cells given a conditioning irradiation in the medium without H-7. The finding indicates that the induction of radio-adaptive response is reduced if the activity of PKC is inhibited by adding 5 mM of H-7. The concentration of the inhibition constant (ki) of H-7 against PKC is 6.0 mM [6]. This supports the previous report demonstrating that intracellular signal transduction related to the radio-adaptive response induced by low-dose irradiation is mediated by PKC [7]. Although the Ki concentration of H-89 against A kinase is 0.048 mM [8] and H-8 against G kinase is 0.48 mM [6], addition of these inhibitors at a concentration corresponding to the Ki concentration, 0.05 mM and 0.5 mM respectively, failed to inhibit the induction of radio-adaptive response. These findings indicate that the intracellular signal transduction for the induction of radio-adaptive response is not mediated by A kinase or G kinase.

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