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Adaptive Response Induced by Low Dose Ionizing Radiation in Human Cervical Carcinoma Cells

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Adaptive response induced by low dose γ-ray irradiation in human cervical carcinoma cells was examined. Cells were exposured to low dose of γ-ray (1 cGy) followed by high doses of γ-ray irradiation (0, 1, 2, 3, 5, 7 and 9 Gy for clonogenic assay or 1.5 Gy for micronucleus assay) with various time intervals. Survival fractions of cells in both low dose-irradiated and unirradiated groups were analyzed by clonogenic assay. Survival fractions of low dose-irradiated cells was higher than that of control group irradiated only with high dose γ-ray. The increase in cell survival was maximum when low and high dose irradiation time interval was 4 hr. Frequencies of micronuclei which is an indicative of chromosome aberration were also enumerated in both low dose-irradiated and unirradiated groups. In consistent with the result obtained from survival fractions analyzed by clonogenic assay, maximum reduction in frequencies of micronuclei was observed when low dose radiation was given 4 hr prior to high dose irradiation. These results demonstrate that low dose γ-ray irradiation induced adaptive response to subsequent high dose γ-ray irradiation in human cervical carcinoma cells. Our data suggest that one of the possible mechanisms of adaptive response induced by low dose radiation is the increase in repair of DNA double strand breaks in low dose radiation-adapted cells.

Key words : Radiation-induced adaptive response, Survival fraction, Micronucleus assay, Gamma-ray, Human cervical carcinoma cells

INTRODUCTION

When bacterial cells (Samson and Cairns, 1977) and mammalian cells (Samson and Schwartz, 1980; Kaina, 1982) are exposed to low doses of alkylating agents, they exhibit adaptive response, that is they are less sensitive to subsequent exposure of the same chemical at a higher dose. It was also observed that a pretreatment of low dose of radiation such as tritiated thymidine (Olivieri *et al.*, 1984; Wiencke *et al.*, 1986) or low doses of X-irradiation (Shadley and Wolff, 1987; Shadley *et al.*, 1987) induced adaptive response, thus resulted in a reduction in the yield of chromosomal aberrations by subsequent high doses of X-rays in human lymphocytes. Such adaptive response induced by low dose radiation were found to occur in the cases of human lymphocytes (Sanderson and Morley, 1986; Wolff *et al.*, 1988; Bosi and Olivieri, 1989; Shadley and Wiencke *et al.*, 1989; Cai and Liu, 1990), animal cells such as Chinese hamster cells (Ikushima, 1987) and mouse embryo cells (Azzam *et al.*, 1994), and plant cells (Cortes *et al.*, 1990).

It is widely known that ionizing radiation directly or indirectly induces damages in biologically important macromolecules in cells. Therefore, the extent of chromosomal aberrations was used as biological end points of radiation damages. Among several analytical methods of chromosomal aberrations, chromatid and isochromatid breaks were used as a biological end points in many cases (Shadley and Wolff, 1987; Shadley *et al.*, 1987; Wolff *et al.*, 1988; Bosi and Olivieri, 1989; Shadley and Wiencke *et al.*, 1989; Cai and Liu, 1990; Cortes *et al.*, 1990). However, since the cytokinesis-blocked micronucleus assay (Fenech and Morley, 1985) has been developed, this method is became employed to measure chromosomal aberrations produced after irradiation of cells because this cytokinesis-blocked micronucleus assay is relatively simple and statistically precise

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method than karyotypic analysis for quantitation of chromosomal damages (Ikushima, 1987; Azzam *et al.*, 1994; Ono *et al.*, 1994). Micronuclei are acentric chromosome fragments or whole chromosome which are not enclosed into main nuclei during cell divisions (Miller *et al.*, 1992).

All of the previous observations of adaptive responses to radiations have been conducted on the normal or normal-driven cell lines. Therefore, it will be important to see if carcinoma cells exhibit adaptive responses to radiations. Furthermore, the data obtained from such studies can be applied to radiotherapy of carcinomas. Cervical carcinoma is one of the most frequent cancers in Korean women, and shows an intermediate radiosensitivity, we used human cervical carcinoma (CaSki) cells in this study. Human cervical carcinoma (CaSki) cells were irradiated with low dose (1 cGy) of γ-ray, and subsequently irradiated with high doses of γ-rays (0, 1, 2, 3, 5, 7 and 9 Gy for cell survival study and 1.5 Gy for micronucleus assay) with time intervals of 4, 7 and 20 hr for cell survival study or 4 and 20 hr for micronucleus assay, and cell survivals and the frequencies of micronuclei were measured to observe induction of adaptive response to ionizing radiation in these cells.

MATERIALS AND METHODS

Cell culture and irradiation

Human cervical carcinoma (CaSki, ATCC CRL 1550) cells were cultured in RPMI1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, and antibiotics in a humidified 5% CO_2/95% air incubator at 37°C. For low dose irradiation, cells were irradiated with 1 cGy of ^{137}Cs γ-ray at a dose rate of 0.143 cGy/min at room temperature. For high dose irradiation, cells were irradiated with 0, 1, 2, 3, 5, 7 and 9 Gy (for clonogenic assay) or with 1.5 Gy (for micronucleus assay) of ^{60}Co γ-rays (Theratron-780 teletherapy unit) at a dose rate of 139.5 cGy/min at room temperature.

Cell survival after irradiation

We employed clonogenic assay (Hall, 1994) to measure cell survival after γ-ray irradiation. Cells were seeded at appropriate cell densities depending on radiation dose to be irradiated in 100 mm plates. Cells were irradiated with low dose radiation followed by high dose irradiation after 4, 7, and 20 hr of incubation period. After 14 days of incubation, cells were fixed in methanol:acetic acid (3 : 1) and stained with trypan blue. Colonies of cells underwent more than 5 cell divisions were counted. Plating efficiencies was about 50% for the cell line used in this study.

Micronucleus assay

Cytochalasin-B (Aldrich Chemical Co.) was added to media at a final concentration of 1 μg/ml immediately after irradiation. After 28 hr incubation in the presence of Cytochalasin-B, cells were trypsinized and fixed as described above. Fixed cells were spread onto slide glasses, air-dried and stained with Giemsa. We used Almassy's method (Almassy *et al.*, 1987) to identify the micronuclei in cytokinesis-blocked binucleated cells and frequencies of micronuclei were scored in 1,000 binucleated cells at 400 X magnification.

RESULTS

Survival fractions and survival curves of human cervical carcinoma (CaSki) cells after γ-ray irradiation are shown in Table I and Fig. 1. When cells were irradiated with high doses of γ-rays, they showed the lowest survivals. We used survival fractions of this group to fit the linear quardratic model. By the linear quardratic model, the expression for the cell survival curve is $SF = Exp-(\alpha D+\beta D^2)$, where SF is the survival fraction of cells at a dose D, and α and β are constants. The following equation was obtained; $SF=Exp-(0.0079D+0.064D^2)$, where α and β values were 0.0079 and 0.064, respectively. When a dose is equal to α/β value, the linear and quardratic contributions to cell killing are equal in this model. When cells were irradiated only with high doses of γ-rays, α/β value was 0.12 Gy. When cells were irradiated with low dose of γ-rays 4, 7, and 20 hr prior to high dose irradiation, the survival rates were increased (Table I and Fig. 1 A-C). Maximum increase in cell survival was observed when cells were irradiated with high and low doses of γ-rays with 4 hr interval (Fig. 1A). When cells were irradiated with high and low doses of γ-rays with 7 and 20 hr intervals, cell survival rates in these groups were also higher than the control group irradiated only with high doses of γ-rays. Again, we used survival fractions of these groups to fit the

Table I. Survival fractions of human cervical carcinoma (CaSki) cells after γ-ray irradiation

Dose (Gy)	Survival Fractions			
	High	4 hr	7 hr	20 hr
0	1.000	1.000	1.000	1.000
1	0.740	0.826	0.771	0.848
2	0.661	0.771	0.741	0.784
3	0.450	0.587	0.504	0.511
5	0.210	0.322	0.250	0.254
7	0.060	0.101	0.006	0.078
9	0.004	0.012	0.005	0.008

*Human cervical carcinoma (CaSki) cells were irradiated with high dose γ-rays (High), or high and low doses of γ-rays with 4 hr (4 hr), 7 hr (7 hr), or 20 hr (20 hr) intervals.

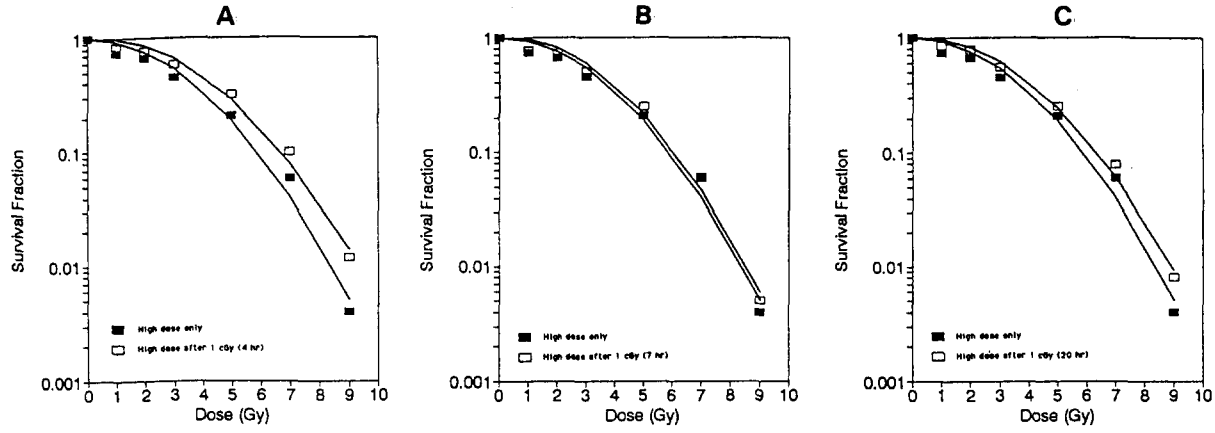

Fig. 1. Survival curves of human cervical carcinoma (CaSki) cells after γ-ray irradiation. Cells were irradiated with 1 cGy of low dose γ-rays 4 hr (A), 7 hr (B) or 20 hr (C) prior to high dose γ-ray irradiation (□) or only with high dose γ-rays (■)

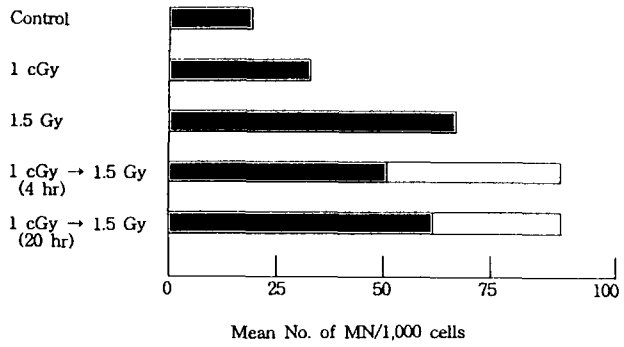

Fig. 2. Micronuclei frequencies in human cervical carcinoma (CaSki) cells irradiated with γ-rays. Filled boxes show frequencies of micronuclei in 1,000 cytokinesis-blocked cells and empty boxes show the expected yields.

linear quardratic model. Equations obtained were $SF = Exp(-0.038D+0.057D^2)$ when irradiation time interval was 4 hr, $SF = Exp(-0.03D+0.067D^2)$ when irradiation time interval was 7 hr, and $SF = Exp(-0.024D+0.061D^2)$ when irradiation time interval was 20 hr. Alpha values of this three survival curves showed negative values. This means that α values of these curves are close to 0 and, therefore, theoretically, α/β values are 0 in these curves. Thus, there is no linear contribution and is only quadratic contributions to cell killing in these survival curves. From the cell survival studies, we have observed that pretreatment of cells with low dose γ-ray irradiation increased cell survivals to subsequent high doses of γ-ray irradiations.

In order to elucidate the possible mechanisms involved in adaptive response induced by low dose γ-ray irradiation, we have investigated if these adaptive responses to radiation also decrease the frequency of chromosome aberration. Cells were irradiated as described above and were cultured for 28 hr in the presence of cytokinesis blocking agent, cytochalasin-B. Then, cells were harvested, fixed and the frequencies of micronuclei was counted in 1,000 binucleated cells. In this experiment, we chose radiation time intervals of 4 hr and 20 hr which exhibited a significant increase in cell survival as described above. Frequencies of micronuclei in human cervical carcinoma cells are shown in Fig. 2. In control group, 17 micronuclei was observed in 1,000 binucleated cells. When cells were irradiated with only 1 cGy or 1.5 Gy, 34 or 71 micronuclei were produced, respectively. Thus, the expected micronucleus frequency in cells irradiated with 1 cGy followed by 1.5 Gy is the sum of the frequencies of micronuclei observed in cells irradiated with 1 cGy or 1.5 Gy, which is 88. However, much lower number of micronucleus was observed in cells irradiated with 1.5 Gy after 1 cGy irradiations. When the irradiation time interval was 4 hr, 52 micronuclei were observed, which is 59.1% of the expected yield. When the time interval was 20 hr, 62 micronuclei were observed which is 70.5% of the expected yield. These data show that when cells were pretreated with 1 cGy of γ-ray irradiation prior to 1.5 Gy of high dose irradiation, they showed reduced production of micronuclei and the reduction of micronuclei was more pronounced when the irradiation time interval was 4 hr. These results in consistent with the results obtained from the cell survival study described above. Our data suggest that one of the mechanisms of the adaptive response induced by low dose radiation is the increase in DNA repair in cells pretreated with low dose of γ-rays, thus, result in the reduction of chromosomal aberrations in these cells.

DISCUSSION

Our data described in this report demonstrate an adaptive response to ionizing radiation exists in human carcinoma cells. Pretreatment of low dose γ-ray irradiation increased cell survivals and reduced micronucleus formation to subsequent high dose γ-ray irradiation in human carcinoma cells. Cells showed

maximum resistance to radiation in cell survival and micronucleus formation when the low dose radiation was given 4 hr prior to high dose irradiation. When the irradiation time interval was 7 hr, they showed the least radioresistance. And they showed the intermediate resistance when the irradiation time interval was 20 hr. Our observations indicate that low dose radiation-induced adaptive response is persist at least 20 hr in human cervical carcinoma cells. Interestingly, cells were less resistant to subsequent radiation when the radiation time intervals was 7 hr. It is possible that this observation has some relation with the control of the cell cycle after irradiation of cells, however, we have no direct evidence at present.

Most important subcellular target of ionizing radiation at biologically relevant doses is the genetic material, DNA. DNA double strand breaks are the critical event in radiation-induced cell killing. Micronuclei are acentric chromosome fragments or whole chromosome produced by DNA double strand breaks (Miller *et al.*, 1992). Since the formation of micronuclei was reduced in radiation-adapted cells, one of the possible mechanism behind this low dose radiation-induced adaptive response is increase in repair of the DNA double strand breaks in these cells. Low dose-irradiated cells show no linear region in their survival curves (Fig. 1). In linear regions of survival curves by the linear quardratic model, the two chromosome breaks are the consequence of a single electron set in motion by the absorption of γ-rays. The probability of an interaction between the breaks is proportional to dose. In quardratic regions, the two chromosomal breaks may result from two separate electrons. The probability of an interaction is then proportional to square of dose. It seems that in the cells adapted to low dose radiation, the repair of DNA double strand breaks are increased, thus in many cases one of two chromosomal breaks are repaired. This results in apparent quardratic regions where the linear regions supposed to be dominant. Our conclusions are supported by other report (Frankenberg-Schwager and Frankenberg, 1994) that showed shoulders of survival curves are the characteristic features of DNA double strand break rejoining. Also, our conclusion of increased DNA double strand break repair in low-dose radiation adapted cells is in good agreement with the results obtained from the micronucleus assay. Similar observation of the relationship between survival curve and micronucleus frequency in cytokinesis-blocked cells was reported (Ono *et al.*, 1994).

It is critically important for clinicians to be able to predict the outcome of radiotherapy, however, carcinomas and normal cells show a wide range of radiosensitivity. We used human carcinoma cells which show intermediate radiosensitivity and revealed these cells exhibited adaptive response to radiation in this study. Our data can be applied to planning of radiotherapy of cervical carcinomas.

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