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Molecular Mechanism of Radioadaptive Response: A Cross-Adaptive Response for Enhanced Repair of DNA Damage in Adapted Cells

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Abstract

The radioadaptive response (RAR) has been attributed to the induction of a repair mechanism by low doses of ionizing radiation, but the molecular nature of the mechanism is not yet elucidated. We have characterize RAR in a series of experiments in cultured Chinese hamster V79 cells. A 4-h interval is required for the full expression of RAR, which decays with the progression of cell proliferation. Treatments with inhibitors of poly(ADP-ribose) polymerase, protein- or RNA synthesis, and protein kinase C suppress the RAR expression. The RAR cross-reacts on clastogenic lesions induced by other physical and chemical DNA-damaging agents. The presence of newly synthesised proteins has been detected during the expression period. Experiments performed using single-cell gel electrophoresis provided more direct evidence for a faster and enhanced DNA repair rate in adapted cells. Here, using single-cell gel electrophoresis, a cross-adaptive response has been demonstrated for enhaced repair of DNA damage induced by neocarzinostatin in radio-adapted cells.

1. Introduction

After cells are pre-exposed to very low-dose radiation, they become less sensitive to subsequent a high dose of radiation for induction of genetic damage. This phenomenon has been termed the radioadaptive response (RAR) [1]. Following the finding of RAR for chromatid aberrations in human lymphocytes [2], we have obseved RAR for the induction of micronuclei and sister-chromatid exchanges in cultured Chinese hamster V79 cells pre-exposed to very low-dose radiation [3]. Thereafter, the RAR has been characterized in a series of our experiments [1, 3, 4, 5]. The RAR can be caused by a narrow window of low doses (cGy level). A 4-h interval is required for the full expression of RAR, which decays rapidly with the progression of cell proliferation. The RAR expression is suppressed by treatments with inhibitors of poly(ADP-ribose) polymerase, protein- or RNA synthesis, and protein kinase C. The RAR cross-reacts on clastogenic lesions induced by other physical and chemical DNA-damaging agents. Proteins are newly synthesised concurrently with the development of the RAR. The similar characteristics have been noticed in human lymphocytes, too [e.g. 6].

These observations suggest that RAR may be attributed to the induction of novel, efficient repair mechanism for chromosomal DNA damage. To verify this hyposesis, we have analysised repair kinetics of DNA double-strnad breaks in adapted cells pre-exposed to low doses using single-cell gel electrophoresis, and provided evidence for a faster and enhanced DNA repair rate in adapted cells [7]. Here, we report the results of experiments performed to ascertain whether RAR cross-reacts for the repair of DNA damage induced by neocarzinostatin, a radiominetic chemical [8].

2. Materials and Methods

Chinese hamster V79 cells were maintained as monolayers in exponential growth by subculturing twice a week in alpha-modified Eagle MEM culture medium (Sigma) supplemented with 10% fetal bovine serum (Bio Whittaker), 20 mM HEPES, 100 U/ml penicillin and 100 μ g/ml streptomycin, at 37 °C in a humidified atomosphere of 95% air/5% CO_2.

The cells cultured to semi-confluence as a monolayer in a tissue culture flask were irradiated with ^{60}Co γ -rays at dose-rate of 5 cGy/min for adapting doses. After the adapting exposure to 5 cGy, the cells were allowed to express fully the adaptive response at 37 °C for further 4 h prior to the challenging treatments with 0.2 U/ml of neocarzinostatin (Kayaku, Japan) for 1 h. The repair kinetics of DNA strand breaks were monitored for 120 min after the neocarzinostatin challenge using single-cell gel electrophoresis. The single-cell gel electrophoresis was performed as described previously [7]. The elongated length of tails was used as semi-quantitative measure of DNA double-strand breaks.

3. Results and Discussion

The neutral gel electrophoresis was applied to the assay of double-strand breaks in chromosomal DNA. The mean length of the elongated comet's tails was increased with increasing concentration of neocarzinostatin, and in the assay immediately after the neocarzinostatin treatments, there was no significant differences in the initial yield of DNA double-strand breaks between non-adapted and adapted cells as shown in Fig. 1. The present data indicate that RAR does not enhance the cellular capacity to suppress the production of initial DNA damge by neocarzinostatin challenge.

To test whether RAR can be explained in terms of DNA repair, the repair kinetics was monitored over 120 min after 0.2 U/ml of neocarzinostatin challenge. As shown in Fig. 2, the rate of rejoining of DNA double-strand breaks induced by neocarzinostatin was higher in adapted cells by pre-exposure to 5 cGy than in non-adapted cells. The enhancement of repair rate was more remarkable in the early phase than the late phase of repair, and less damage remained in adapted cells than non-adapted cells. The results indicate that RAR caused by pre-exposure to low-dose radiation may cross-react on chromosomal DNA lesions produced by neocarzinostatin for enhanced DNA repair.

We have demonstrated that pre-exposure to low doses results in a faster and enhanced repair of DNA damage induced by a subsequent high dose of radiation. From the present results, it is inferred that pre-exposure to low-dose radaition may bring about a faster and enhanced repiar of DNA damage, at least double-strand breaks, induced by subsequent treatments with DNA damaging agents other than radiation as well. For the induction of cytogenetical damage, such a cross-adaptive responses have been reported in several combinations of adapting and challenging treatments [4, 9].

The RAR is effectively suppressed by 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase, which plays an important role in the rejoining of DNA strand breaks [1]. The RAR requires 4 to 6 h to express fully [5, 10], and this response accompanies de novo protein synthesis during this period [5, 11]. The development of RAR is also blocked by protein kinase C inhibitor [5]. These facts suggest a possible intracellular process of RAR that pre-exposure to low-dose radiation generates a signal of small amount of DNA damage, and the signal, through a signal transduction pathway mediated by protein kinase C, is sent back to the nucleus. This chain of events may trigger the activation of a set of genes encoding repair enzymes and/or factors leading to de novo protein synthesis, and enhance the repair capacity of

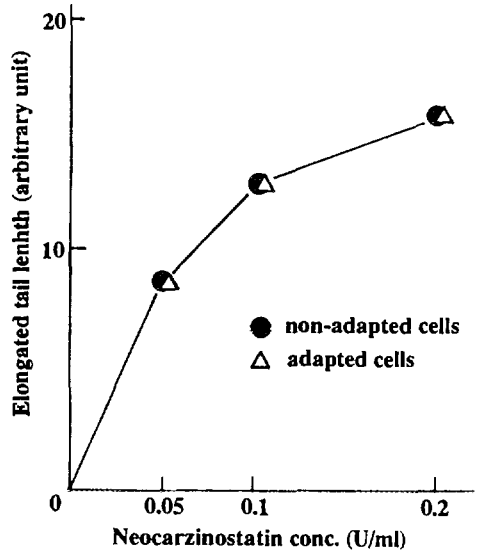

Fig. 1. Induction of DNA double-strand breaks by neocarzinostatin in adapted and non-adapted cells.

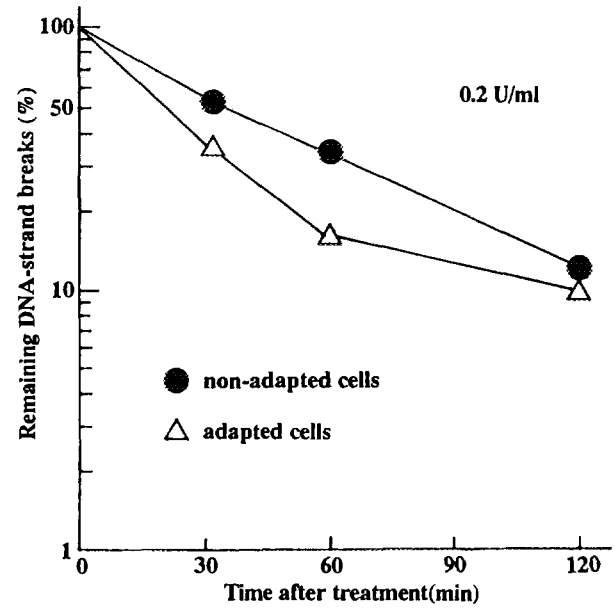

Fig. 2. Repair kinetics of DNA double-strand breaks induced by neocarzinostatin in adapted and non-adapted cells.

the serious DNA damage induced by a subsequent challenge by radiation or other mutagens as depicted in the previous papaer [7]. It is intresting whether Ku protein and DNA-PK are involved in this process [12]. This hypothsis is worthy to be explored further.

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