Mutation Research 773 (2014) 34–38

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Role of DNA methylation in the adaptive responses induced in a human B lymphoblast cell line by long-term low-dose exposures to γ-rays and cadmium

Shuang Ye[a,b], Dexiao Yuan[a], Yuexia Xie[a], Yan Pan[a], Chunlin Shao[a,*]

[a] Institute of Radiation Medicine, Fudan University, No. 2094, Xietu Road, Shanghai 200032, China
[b] Ningbo Medical Center, Lihuili Hospital, No. 57, Xingning Road, Ningbo 315041, Zhejiang, China

ARTICLE INFO

Article history:
Received 13 June 2014
Received in revised form 19 August 2014
Accepted 25 August 2014
Available online 3 September 2014

Keywords:
Low-dose radiation
Low-dose cadmium
Adaptive response
Lymphoblast
DNA methylation

ABSTRACT

The possible involvement of epigenetic factors in health risks due to exposures to environmental toxicants and ionizing radiation is poorly understood. We have tested the hypothesis that DNA methylation contributes to the adaptive response (AR) to ionizing radiation or Cd. Human B lymphoblast cells HMy2.CIR were irradiated (0.032 Gy γ-rays) three times per week for 4 weeks or exposed to $CdCl_2$ (0.005, 0.01, or 0.1 μM) for 3 months, and then challenged with a high dose of Cd (50 or 100 μM) or γ-rays (2 Gy). Long-term low-dose radiation (LDR) or long-term low-dose Cd exposure induced AR against challenging doses of Cd and irradiation, respectively. When the primed cells were treated with 5-aza-2′-deoxycytidine (5-aza-dC), a DNA methyltransferase inhibitor, the ARs were eliminated. These results indicate that DNA methylation is involved in the induction of AR in HMy2.CIR cells.

1. Introduction

Evaluation of the biological effects of low-dose radiation (LDR) on the basis of the linear-non-threshold (LNT) model; extrapolating from high-dose results; is of questionable validity [1]. Cellular responses to LDR; including the adaptive response (AR); genomic instability; hypersensitivity; and the bystander effect; differ from the response to high-dose radiation [2–5]. The radioadaptive response (RAR); cellular resistance against subsequent challenging radiation; is an important effect of LDR. Several factors; including DNA repair; cell cycle regulation; antioxidant defense; and the suppression of p53 accumulation; may participate in regulating the RAR [6,7]. The AR can also be induced by genotoxic agents; such as inorganic ions; including Cd; Cu; Zn; and Se [8–10].

Cadmium is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and the National Toxicology Program, U.S.A. [11,12]. Expression of several stress-response genes, including those encoding heat-shock proteins, glutathione biosynthesis enzymes, and metallothionein (MT) have been implicated in Cd-induced AR [13–15]. Since long-term LDR and low-dose Cd are widespread, health risks due to environmental factors should be assessed in the context of multiple exposures. However, the underlying mechanisms of such combination effects are unclear.

DNA methylation, an epigenetic factor, is important for cell proliferation, development, gene expression, and maintenance of genome stability in many organisms, both prokaryotic and eukaryotic [16–18]. LDR responses, such as genomic instability and the bystander effect, might be regulated by DNA methylation [1,19,20]. Our previous study showed that global genomic DNA hypermethylation might be involved in the long-term LDR-induced AR against a high challenge dose of radiation [21].

DNA methylation can also be facilitated by long-term exposure to Cd [22–25]. Benbrahim-Tallaa et al. found that DNA hypermethylation at the global and gene-specific levels occurs in association with Cd-induced malignant transformation [23]. Growing evidence suggests that some responses of LDR and Cd may be regulated by DNA methylation, yet the mechanisms remain to be elucidated.

In the present study, using long-term LDR and long-term low-dose Cd treatments of human lymphoblast cells, we examined the AR induced by these two stresses and investigated the possible underlying role of DNA methylation in these responses. A deeper insight into the molecular mechanism of AR induced by physical and chemical factors may improve the knowledge of cancer risk evaluation of environmental toxicants.

Abbreviations: LDR, low-dose radiation; AR, adaptive response; MAR, magnitude of adaptive response; 5-aza-dC, 5-aza-2′-deoxycytidine; CB, cytochalasin B; MN, micronucleus.

* Corresponding author. Tel.: +86 21 64048677; fax: +86 21 64048677.
E-mail address: clshao@shmu.edu.cn (C. Shao).

http://dx.doi.org/10.1016/j.mrgentox.2014.08.004
1383-5718/

2. Materials and methods

2.1. Cell culture

An immortalized but non-tumorigenic human B lymphoblast cell line, HMy2.CIR, was obtained from Shanghai Cell Bank (Shanghai, China) and cultured in Iscove's modified Dulbecco's medium (IMDM; HyClone, Beijing, China) containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37 °C with 5% CO_2.

2.2. Priming and challenging treatments

HMy2.CIR cells were seeded at 5×10^5 cells/dish, maintained overnight, and then irradiated with γ-rays using a ^{137}Cs source (Gammacell-40, MDS Nordion; Ottawa, Canada) at a dose rate of 0.78 Gy/min. For the long-term LDR group, cells were irradiated with 0.032 Gy per exposure, Monday, Wednesday, and Friday of each week for 4 weeks. The control group was sham irradiated. 4 h after the last irradiation, the primed cells and controls were further challenged with 2 Gy γ-rays or treated with 50 or 100 μM Cd for 1 h.

For long-term Cd treatment, $CdCl_2$ (99%) was applied. The toxic effect of Cd was tested by a cell proliferation assay. Cells were treated with Cd for 1 h and then proliferation was measured with a CCK-8 kit (Dojindo, Kumamoto, Japan) and the half-maximal inhibitory concentration (IC_{50}) was calculated. For the priming treatment with Cd, cells were cultured in medium containing 0, 0.005, 0.01, or 0.1 μM Cd for 3 months; then the Cd-primed cells were further exposed to a challenge dose (2 Gy) of γ-rays or high doses (50 or 100 μM) of Cd.

2.3. Micronucleus assay

Micronuclei (MN) were used as a biological endpoint for the evaluation of AR and also as an indicator of radiation-induced genomic instability, and measured with the cytokinesis-block technique [26]. In brief, cells were treated with cytochalasin B (CB, Sigma, USA; 3 μg/ml for 30 h) followed by hypotonic treatment (0.075 M KCl) for 15 min, and then fixed with methanol:acetic acid (9:1, v/v) overnight. The fixed cells were dropped onto pre-chilled glass slides, air-dried, and then stained with Giemsa for 30 min. At least 1000 binucleated cells with well-defined cytoplasm were counted according to the scoring criteria of the MN assay [26]. The MN yield, Y_{MN}, was calculated as the ratio of the number of MN to the number of binucleated cells.

The following equation was used to evaluate the magnitude of AR (MAR):

$$\mathrm{MAR} = \frac{Y_{\mathrm{MN}} \text{ of cells exposed to challenging dose alone}}{Y_{\mathrm{MN}} \text{ of cells exposed to priming and challenging doses}} \quad (1)$$

MAR >1 shows a protective effect of the priming treatment against the challenge, and a larger MAR value indicates a greater efficiency of AR. Each experiment has its own MAR, and the mean and standard error of MAR from at least three experiments were calculated.

2.4. 5-Aza-2′-deoxycytidine (5-aza-dC) treatment

To measure the effect of DNA methylation on AR, cells were treated with 1 μM 5-aza-dC (Sigma) for 72 h to inhibit global DNA methylation [21]. After long-term low-dose treatment with γ-rays or Cd, the primed cells were reseeded at 5×10^5 cells/dish and cultured for 72 h in IMDM containing 1 μM 5-aza-dC; medium was replaced every 24 h. After 5-aza-dC treatment, the primed cells were further challenged with radiation or Cd to detect whether AR would still be induced.

2.5. Statistical analysis

Means and standard errors (S.E.) were calculated for all data from at least three replicate experiments. The means were compared between samples by the Student's *t*-test analysis using SPSS 17.0 software. $P < 0.05$ was considered significant.

3. Results

3.1. Long-term LDR induced AR

γ-Irradiation with a single dose less than 0.1 Gy always induces AR [27,28]. To test whether AR could be induced in HMy2.CIR cells by long-term LDR of γ-rays, after the last exposure of γ-rays, the LDR-primed cells and sham-irradiated control cells were further treated with a challenge dose of γ-rays (2 Gy) or Cd (50 or 100 μM). Our previous studies showed that this long-term LDR did not cause obvious DNA damage, but 2 Gy γ-rays increased the yield of MN to 0.08. However, when the cells were primed with long-term LDR and then challenged with 2 Gy irradiation, the yield of MN was significantly lower than for naive cells, only about 0.05 [21], indicating that AR is induced by the long-term LDR. Here, 50 μM Cd treatment increased the yield of MN to 0.1; but when the cells were primed with long-term LDR and then challenged with 50 μM Cd, the yield of MN was significantly lower, about 0.066 (Fig. 1), indicating radiation-induced AR against Cd challenge. However, when the LDR-primed cells were further challenged with 100 μM Cd, MN induction was only slightly ($P > 0.05$) lower than that of naive cells. Perhaps the cells were seriously damaged by treatment with 100 μM Cd, so that no obvious AR was induced by the LDR.

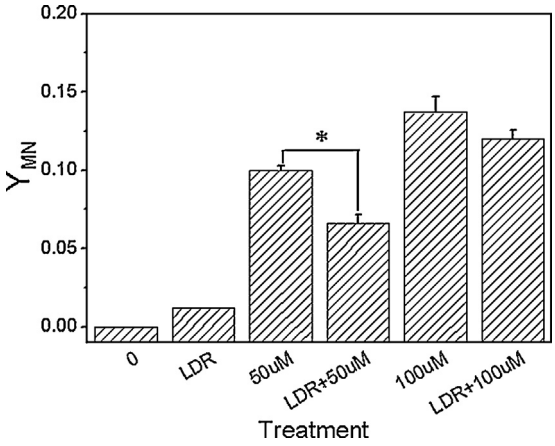

Fig. 1. MN induction in HMy2.CIR cells. Cells were irradiated with 0.032 Gy γ-rays thrice weekly for 4 weeks and then challenged with 50 or 100 μM Cd. *$P < 0.05$ between the indicated groups.

3.2. Long-term low-dose Cd induced AR

To test the toxicity of Cd, cells were treated with Cd for 1 h and then viability was measured with a CCK-8 kit. The dose-response curve is shown in Fig. 2. The IC_{50} value for Cd = 76.9 μM. Cd at a concentration below 10 μM had no obvious toxic effect (MN formation). Thus, we selected a concentration lower than 1.0 μM as the priming dose for long-term Cd treatment.

When cells were cultured in medium containing 0.005, 0.01, or 0.1 μM Cd for 3 months, no obvious DNA damage was observed. When these primed cells were further challenged with 50 μM Cd, MN induction was significantly lower than that for naive 50 μM Cd treatment (Fig. 3A); hence, AR was triggered by long-term low-dose Cd exposure. However, when these Cd primed-cells were challenged with 100 μM Cd, no AR phenomenon was observed. On the other hand, when these Cd primed-cells were further challenged with 2 Gy γ-rays, MN yields were obviously lower that that for 2 Gy irradiation alone (Fig. 3B). Accordingly, the long-term low-dose Cd

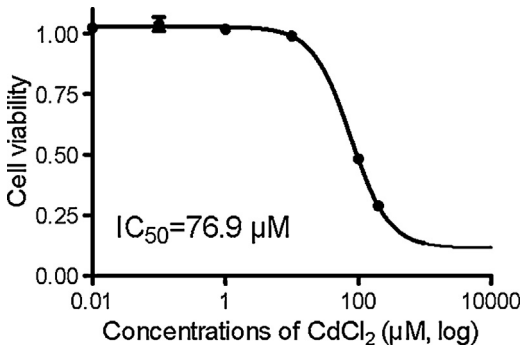

Fig. 2. Cytotoxic effect of Cd on cell viability. HMy2.CIR cells were treated with Cd for 1 h and proliferation was measured with a CCK-8 kit. Cd IC_{50} was calculated with the software GraphPad Prism 5.