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Cadmium induced radioadaptive response via an ATM-independent H_2S/cystathionine γ-lyase modulation

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ABSTRACT

The combined exposure to environmental toxicants such as heavy metals and radiation is an important research area in health protection. Here we explored cadmium induced radioadaptive response (RAR) and investigated the role of hydrogen sulfide (H_2S) and ATM kinase in this response. Our data showed that the cadmium ions with a sub-lethal concentration could induce RAR in Chang liver cells towards subsequent γ-irradiation and this response could be abrogated by DL-propargylglycine (PPG), the endogenous H_2S synthetase inhibitor of cystathionine γ-lyase (CSE), but not by aminooxyacetic acid (AOAA), the inhibitor of cystathionine β-synthase (CBS). Moreover, the pretreatment of cells with NaHS also stimulated cellular adaptive response to radiation. Both cadmium treatment and irradiation up-regulated the expression of CSE protein in a time-dependent manner but had no influence on the expression of CBS protein. In the primed cells, the time course of CBS expression showed no significant difference with the cells treated with 2Gy irradiation alone, however, the CSE expression was easier to reach the maximum level, indicating a more efficient H_2S production by CSE. Moreover, the cadmium-induced RAR was totally suppressed by KU-55933, a specific ATM inhibitor that did not change the CSE expression after radiation. However, exogenous H_2S decreased the phosphorylation level of radiation-induced ATM. In conclusion, the present results demonstrate firstly that H_2S is involved in the cadmium induced cross-adaptive response to challenging radiation. CSE, rather than CBS, may mainly responsible for the H_2S production during this RAR which may also be mediated by ATM pathway. However, the activation of CSE is independent of ATM but could negatively regulate the phosphorylation of ATM.

1. Introduction

The radioadaptive response (RAR) is a phenomenon where cells pretreated with a sub-genotoxic low dose of a mutagen become significantly resistant to a challenging radiation exposure. Many relative reports have been published since 1980s, and the RAR involves probably the transcription of many genes and the activation of numerous signaling pathways that trigger cell defenses such as more efficient detoxification of free radicals [1,2], enhancement of antioxidant production [3], induction of new proteins [4], and activation of DNA repair system and cell cycle regulation [5]. Recently, oxidative stress induced by low dose of radiation was demonstrated to be a significant factor for the development of RAR [6]. However, the underlying mechanisms, especially the signal transduction involved in the RAR remain largely unknown. Moreover, more information is needed for cross adaptation. Because human health is threatened not only by radiation but also by environmental chemical toxicants, thus the health risk caused by environmental factors should be assessed in the context of combined exposure. Cross adaptation and synergistic sensitization may play important roles in modifying the biological effect caused by environmental factors to which we are exposed in our daily life.

Cadmium is one of the most frequent environmental toxins. The wide use in industry and persistence in environment make cadmium one of the hot spots in the toxicology research field. Cadmium is only weakly genotoxic but it can induce multiple cellular dysfunctions such as aberrant gene expression and signal transduction, interference with enzymes of the cellular antioxidant system and enhanced generation of reactive oxygen species (ROS), inhibition of DNA repair and DNA methylation (for reviews, see Ref. [7–9]). It was reported that cadmium decreased the repair rate of X-ray-induced DSBs by affecting DNA-PK kinase activity [10]. The combined action of ionizing radiation and cadmium could lead to increased DNA damage formation compared to the effects of individual stressors [11,12]. However, it was also reported that the combined expo-

Abbreviations: RAR, radioadaptive response; PPG, DL-propargylglycine; AOAA, aminooxyacetic acid; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; KU-55933, 2-morpholin-4-yl-6-thianthren-1-yl-pyran-4-one; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; MN, micronuclei; ATM, ataxia-telangiectasia mutated; ROS, reactive oxygen species.

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sure to $CdCl_2$ and X-rays led to a significant reduction in the rate of exencephaly compared with the high frequency after cadmium exposure alone [13], and cadmium has protective effect both *in vivo* and *in vitro* against radiation [14–16].

Hydrogen sulfide (H_2S) was once viewed as an environmental contaminant and was thought to be toxic to human health. However, it is now becoming increasingly clear that this gas can also be synthesized naturally in many mammalian tissues and evidence has accumulated that H_2S may play a number of physiological functions, serve as a novel third gastransmitter in the body alongside nitrogen oxide (NO) and carbon monoxide (CO) [17]. In this respect, recent studies have focused on the role of this gas in physiology, such as the cardiovascular system inflammation [18,19], and receptor-mediated responses in neurons [20]. H_2S is endogenously synthesized in various mammalian tissues by two pyridoxal-5-phosphate-dependent enzymes responsible for metabolizing L-cysteine, cystathionine β-synthase (CBS, EC 4.2.1.22) and cystathionine γ-lyase (CSE, EC 4.4.1.1) [21,22]. Physiological concentrations of this gas in plasma have been reported between 20 and 160 μM [23,24]. Previous studies have shown that H_2S can modulate cellular proliferation and apoptosis via MAPK pathways, regulate cell cycle-related proteins such as cyclin D1, p21 [25], and that H_2S exerts some physiological functions by opening K_{ATP} channels [23]. However, up to our knowledge, there is little literature concerning the relationship between H_2S and RAR. Here, we investigated the cadmium-induced RAR and the role of H_2S in this response, as well as the possible interaction between H_2S and ATM.

2. Materials and methods

2.1. Cell line and culture

Chang liver cells, originated from human normal liver tissue, were purchased from Shanghai Cell Bank and maintained in DMEM medium (Gibco, Hangzhou, China) with 10% fetal bovine serum (Gibco, Invitrogen, USA), 2 mM L-glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin and cultured in a humidified atmosphere of 5% CO_2 in air at 37 °C. The cells were propagated every three days and reseeded at 6×10^5 cells per T25 flask.

2.2. Cadmium, H_2S treatment and irradiation

Cadmium chloride ($CdCl_2$, Sinopharm Chemical Reagent Co. Ltd., China) was dissolved in ddH_2O and sterilized through a 0.22 μm filter to have a stock solution which was diluted with culture medium to the desired concentrations for cell treatment. NaHS (Sigma, USA) was used as the donor of H_2S. In aqueous solution, NaHS can be decompounded to Na^+ and HS^-, and HS^- further associate with H^+ to generate stable H_2S. Approximately one third of H_2S is in the non-dissociated form.

Cells (1.5×10^5 cells/dish) were seeded and grown for 24 h to allow cell attachment and then treated with $CdCl_2$ at a priming dose of 1 μM. After 1 h of this priming treatment, the cells were washed twice with pre-warmed phosphate-buffered saline (PBS), incubated in fresh medium for 4 h, and then challenged with 2Gy of γ-rays at a dose rate of 0.8Gy/min. A Cs-137 instrument (Gammacell-40, MDS Nordion, Canada) was used as the γ-ray irradiation source. For H_2S treatment, cells were exposed to 50 or 75 μM NaHS in DMEM for 5 h before 2Gy challenging radiation. Then, these cells were further treated for micronuclei (MN) assay. To examine the adaptive response, parallel experiments were also performed, where cells did not receive any priming cadmium treatment prior to the exposure of radiation.

2.3. Treatment of inhibitors

2-Morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU-55933) (Calbiochem, Germany), a specific inhibitor for ATM, was dissolved in dimethyl sulfoxide (DMSO), DL-propargylglycine (PPG, an inhibitor of CSE) or aminooxyacetic acid (AOAA, an inhibitor of CBS) was dissolved in PBS and stored at −20 °C as stock solutions (10 mM for KU-55933, 400 mM for PPG, 100 mM for AOAA). The working concentration was 2 mM for PPG, 1 mM for AOAA, and 10 μM for KU-55933.

For KU-55933 treatment, cells were divided into six groups: group "control" (cells did not receive any radiation or cadmium treatment), group "K" (cells were treated with 10 μM KU-55933 for 5 h), group "1 + 2Gy" (cells were treated with 1 μM $CdCl_2$ for 1 h, washed twice with PBS, cultured for 4 h and then challenged with 2Gy γ-radiation), group "2Gy" (cells were exposed to 2Gy γ-radiation), group "1 + 2Gy + K" (cells were treated with 1 μM $CdCl_2$ and 10 μM KU-55933 for 1 h, washed twice with PBS, changed to medium containing 10 μM KU-55933 for 4 h and then challenged with 2Gy γ-radiation), and group "K + 2Gy"(cells were treated with 10 μM KU-55933 for 5 h and then exposed to 2Gy γ-radiation). For all six groups, 0.1% (v/v) DMSO was always contained in the medium as a control of KU-55933 treatment.

For PPG or AOAA treatment, cells were divided into four groups and subjected to the following treatments: group "control" (cells did not receive any treatment), group "inhibitor" (PPG or AOAA treatment alone), group "Cd + inhibitor + 2Gy" (cells were treated with 1 μM $CdCl_2$ and 2 mM PPG or 1 mM AOAA for 1 h, washed twice with PBS, changed to medium containing 2 mM PPG or 1 mM AOAA for 4 h and then challenged with 2Gy γ-radiation), and group "2Gy" (cells were exposed to a single 2Gy γ-radiation).

2.4. Micronuclei assay

MN were used as a biological endpoint for adaptive response and measured with cytokinesis-block technique. Briefly, the cells were exposed to1 μg/ml cytochalasin-B (Sigma, USA) for 30 h and then fixed *in situ* with methanol:acetic acid (9:1 v/v) for 30 min. Air-dried cells were stained with 0.01% acridine orange for 5 min. MN was scored in at least 500 binucleated cells. The MN yield, Y_{MN}, is the ratio of the number of MN to the number of binucleated cells scored.

2.5. Western blot analysis

Cultured cells (2×10^6) were harvested after the indicated treatments (as described in Sections 2.2 and 2.3), washed three times with pre-cold PBS on ice and lysed with RIPA lysis buffer (Beyotime Inst. Biotechology, Haimen, China) according to the manufacturer's instruction. The extracts were denatured at 95 °C for 10 min and centrifuged at 12,000 rpm for 5 min at 4 °C. The supernatants were then collected and stored in −80 °C until use. For Western blotting, equal amounts of protein were loaded per lane and resolved on a 10% (for CSE, CBS) or 8% low-bis (for p-ATM) SDS-PAGE gel, transferred onto 0.22 μM polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore Corporation). Membranes were blocked for 1 h with 5% skim milk in 0.05% Tris-buffered saline/Tween (TBST) and then incubated with the primary antibody (diluted 1:1000 in blocking buffer for CSE (Protein Tech Group, Chicago, USA), 1:500 for CBS (Abcam, Cambridge, UK), 1:1000 for p-ATM (Ser-1981) (Cell Signaling Technology, Boston, USA), and 1:1000 for β-actin (Beyotime Inst. Biotechology, Haimen, China)) at room temperature for 2–3 h or 4 °C overnight. The membranes were then washed, incubated with HRP-conjugated anti-mouse IgG or anti-rabbit IgG (1:2500) and detected by enhanced chemiluminescence system (Beyotime Inst. Biotechology, Haimen, China). The protein image was recorded by the BIO-RAD ChemiDoc XRS and analyzed using the Quantity One software (Bio-Rad, Hercules, CA, USA).

2.6. Statistics

The data were compiled from at least three independent experiments with 3–4 replicates each case and expressed as means ± SE. Data were analyzed with the Student's *t*-test and $P < 0.05$ was considered as significant difference between samples with and without priming treatment or between samples of test and control.

3. Results

3.1. Cadmium-induced RAR and the influence of PPG and AOAA on this response

To know whether radioadaptive response could be induced by cadmium in Chang cells, the cells were pretreated with 0.1 or 1 μM cadmium and subsequently exposed to 2Gy irradiation with 4 h interval between two treatments. As shown in Fig. 1A, both cadmium pretreatments had no obvious toxic effect on DNA damage but they significantly protected the cells against MN formation induced by irradiation ($P < 0.01$), and the magnitude of adaptive response were similar for these two priming doses of cadmium. Here we applied 1 μM as the priming dose for the following cadmium experiments.

To investigate the potential factor involved in this adaptive response, we treated the cells with PPG and AOAA, inhibitor of CSE and CBS respectively. Our results showed that PPG or AOAA alone had no significant effect on the induction of MN in the cells compared with the control. However, the cadmium induced RAR was totally suppressed by the addition of PPG while it was not suppressed by AOAA (Fig. 1B), suggesting that the CSE-related H_2S induction may contribute to the cadmium-induced RAR.