Biochemical and Biophysical Research Communications 445 (2014) 95–99

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cadmium induces cytotoxicity in human bronchial epithelial cells through upregulation of eIF5A1 and NF-kappaB

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ARTICLE INFO

Article history:
Received 21 January 2014
Available online 31 January 2014

Keywords:
Cadmium
BEAS-2B
eIF5A1
NF-kappaB
Cell death

ABSTRACT

Cadmium (Cd) and Cd compounds are widely-distributed in the environment and well-known carcinogens. Here, we report that in $CdCl_2$-exposed human bronchial epithelial cells (BEAS-2B), the level of p53 is dramatically decreased in a time- and dose-dependent manner, suggesting that the observed Cd-induced cytotoxicity is not likely due to the pro-apoptotic function of p53. Therefore, this prompted us to further study the responsive pro-apoptotic factors by proteomic approaches. Interestingly, we identified that high levels (20 or 30 μM) of Cd can significantly upregulate the protein levels of eukaryotic translation initiation factor 5A1 (eIF5A1) and redox-sensitive transcription factor NF-κB p65. Moreover, there is an enhanced NF-κB nuclear translocation as well as chromatin-binding in Cd-treated BEAS-2B cells. We also show that small interfering RNA-specific knockdown of eIF5A1 in Cd-exposed cells attenuated the Cd cytotoxicity, indicating the potential role of eIF5A1 in Cd cytotoxicity. As eIF5A1 is reported to be related with cell apoptosis but little is known about its transcriptional control, we hypothesize that NF-κB might likely modulate *eIF5A1* gene expression. Notably, by bioinformatic analysis, several potential NF-κB binding sites on the upstream promoter region of *eIF5A1* gene can be found. Subsequent chromatin immunoprecipitation assay revealed that indeed there is enhanced NF-κB binding on *eIF5A1* promoter region of Cd-treated BEAS-2B cells. Taken together, our findings suggest for the first time a regulatory mechanism for the pro-apoptotic protein eIF5A1 in which its level is possibly modulated by NF-κB in human lung cells.

1. Introduction

Cadmium (Cd) and Cd compounds are widely-distributed in the living environment. In particular, the contamination of Cd in the food chain, consumption of tobacco and occupational exposure in industry provide the primary sources of human exposure to Cd [1–3]. Cd is toxic, and it has been shown that Cd can cause a variety of adverse health effects, including various types of lung diseases and cancers [1,3]. As a matter of fact, Cd and Cd compounds have been classified as group 1 carcinogens by the International Agency for Research on Cancer (IARC) [3]. The most obvious correlation between Cd and human diseases is found in the lungs [1,4,5]. The mechanism has, however, not been well-established. Evidence has indicated that reactive oxygen species (ROS) may be involved in Cd toxicity and carcinogenicity [6–8].

Our previous studies reported the characterization of Cd exposure in our established normal rat lung epithelial cells (LEC) which showed that Cd is able to exert oxidative stress-induced cytotoxicity [9,10]. Nevertheless, the cellular response would be more reminiscent to human situation if human lung cells are to be used. For this reason, in this study, we resolved to use the normal human bronchial epithelial cells (BEAS-2B) to examine the cellular response to environmentally-relevant concentrations of Cd.

By using normal BEAS-2B cells to simulate the cellular response of human lung cells to Cd, we reported that while a low level (2 μM) of Cd treatment for 36 h elicited negligible cytotoxicity, however, high levels (20 or 30 μM) of Cd treatment for 36 h induced cell death in BEAS-2B cells. Interestingly, we identified that high levels of Cd can upregulate the protein levels of eukaryotic translation initiation factor 5A-1 (eIF5A1) and redox-sensitive transcription factor NF-κB p65. The pro-apoptotic role of eIF5A1 that played in Cd cytotoxicity has been supported by the fact that small interfering RNA-specific knockdown of eIF5A1 in Cd-exposed

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http://dx.doi.org/10.1016/j.bbrc.2014.01.146
0006-291X/

cells attenuated the Cd cytotoxicity. Notably, by bioinformatic analysis and chromatin immunoprecipitation assay, we revealed that there is enhanced NF-κB binding on *eIF5A1* promoter region of Cd-treated BEAS-2B cells. Taken together, our findings suggest for the first time a regulatory mechanism for the pro-apoptotic protein eIF5A1 in which its level is possibly modulated by NF-κB in human lung cells.

2. Materials and methods

2.1. Materials

Cadmium chloride ($CdCl_2$) was purchased from Sigma Aldrich (St. Louis, MO). PlusOne 2-D Clean-Up kit and Silver Staining kit were purchased from GE Healthcare (Uppsala, Sweden). The Subcellular Protein Fractionation Kit for Cultured Cells was from Thermo Scientific (Rockford, IL). siRNAs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). eIF5A1 siRNA (h) is a pool of 4 target-specific 19-25 nt siRNAs designed to efficiently knock down *eIF5A1* gene expression. Control siRNA-A is a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. All other general chemicals were purchased from GE Healthcare and Sigma Aldrich. Antibodies used for Western blot were purchased from Santa Cruz Biotechnology, Sigma Aldrich, Cell Signaling Technology (Danvers, MA) and GeneTex (Irvine, CA).

2.2. Cell culture and transfection

The human bronchial epithelial cell line (BEAS-2B) was purchased from the American Type Culture Collection (ATCC) (Rockville, MD). BEAS-2B cells were isolated from normal human bronchial epithelium obtained from autopsy of a non-cancerous individual. Cells were routinely grown in LHC-9 medium (Gibco, Grand Island, NY) at 37 °C in an atmosphere of 5% CO_2/95% air as recommended by ATCC. BEAS-2B cells were cultured in six-well plates before transfection with small interfering RNA (siRNA) duplexes against eIF5A1 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). At 36 h after transfection, the cells were harvested for Western blot analysis or challenged with $CdCl_2$. Control experiments were carried out with the control siRNA-A under the conditions of eIF5A1 siRNA.

2.3. Cd treatment

Cells were grown to 75% confluence and then were either sham-exposed or treated with different concentrations of $CdCl_2$. Cell viability was measured by naphthol blue black (NBB) staining assay as described previously [11].

2.4. Cell lysate preparation and conditions of Western blot

After treatment, cells were then washed thrice with ice-cold PBS, scraped into centrifuge tube, and then harvested by centrifugation at 1000*g* for 5 min at 4 °C. For subcellular proteins preparation, the cytoplasmic, nuclear-soluble, and chromatin-bound fractions were prepared using the Subcellular Protein Fractionation Kit for Cultured Cells in accordance with the manufacturer. For Western blot analysis, cell pellets were lysed in radioimmunoprecipitation assay buffer according to the protocol as described previously [10]. Equal amounts of proteins (40 μg) were fractionated on a SDS–polyacrylamide gel and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk in PBS containing 0.05% Tween 20 and probed with various primary antibodies. After incubation with secondary antibodies, immunoblots were visualized with the enhanced chemiluminescence detection kit (GE Healthcare).

2.5. Definition of transcription factors binding sites based on Position Weight Matrix (PWM) in eIF5A1 promoter

The whole *eIF5A1* gene and upstream 1500 bp sequences were downloaded from GeneBank. The DNA sequence was analyzed using the canonical Position Weight Matrix (PWM) of NF-κB reported in Jaspar database (http://jaspar.genereg.net/) [12]. Affinity scores were assigned to each promoter binding site using a standard log-likelihood ratio (LLR) scoring functions with intergenic background frequencies. All sites with score exceeding 80% recommended by the PWM were selected as the putative binding sites.

2.6. ChIP-PCR assay

ChIP assays were performed using the ChIP kit (Abcam, Cambridge, MA) in accordance with the manufacturer. Primers that target NF-κB elements are located around the proximal promoter and the first intron of *eIF5A1* gene, primer sequences are listed in Supplementary Table 1.

2.7. Statistical analysis

Statistical analysis was done by using two-tailed Student's *t* test, and $P < 0.05$ was considered significant. Data are expressed as the mean ± SD of triplicate samples, and the reproducibility was confirmed in three separate experiments.

3. Results

3.1. Cytotoxicity of Cd in BEAS-2B cells

Since different types of cell lines would have different sensitivity to Cd exposure, to compare the sensitivity of BEAS-2B cells to Cd, cells were treated with varying concentrations of $CdCl_2$ and cell viability was determined by NBB assay. Increasing Cd concentrations exhibited cytotoxicity to BEAS-2B cells. Cell viability is unaffected at 1 or 2 μM $CdCl_2$ but severely-compromised at higher concentrations of $CdCl_2$ (Fig. 1). From the data, the lethal concentration range is around 20–30 μM, and we therefore used these dosages for subsequent experiments.

3.2. The effects of Cd exposure on p53 levels in BEAS-2B cells

As shown in Fig. 1, high levels of Cd induced cell death in BEAS-2B cells. We first examined whether the cell death is mediated by the pro-apoptotic functions of tumor suppressor p53. Interestingly, the level of p53 is downregulated in a time- and dose-dependent manner (Supplementary Fig. 1). We also checked the levels of Bid and PARP, which indicated the time-dependent Bid and PAPR cleavage, supporting the cell death observed in BEAS-2B is triggered by apoptotic cascades (Supplementary Fig. 2). Since the observed Cd-induced cytotoxicity is not likely due to the pro-apoptotic function of p53. Therefore, this prompted us to further study the responsive pro-apoptotic factors by proteomic approaches.

3.3. Identification of eIF5A1 as a Cd-responsive protein by proteomic approach

In order to identify the Cd-responsive proteins which might promote cell death, to this end, 2D-PAGE proteome analysis was conducted on sham-exposed and $CdCl_2$-treated BEAS-2B cells. Spots that displayed significantly differences were cut out and