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

De-Ju Chen [a,b,1], Yan-Ming Xu [a,b,1], Ji-Ying Du [a,b], Dong-Yang Huang [b], Andy T.Y. Lau [a,b,*]

[a] Laboratory of Cancer Biology and Epigenetics, Shantou University Medical College, Shantou, Guangdong 515041, China
[b] Department of Cell Biology and Genetics, Shantou University Medical College, Shantou, Guangdong 515041, China

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

* Corresponding author. Address: Room 603, Comprehensive Building, Shantou University Medical College, 22 Xinling Road, Shantou, Guangdong 515041, China. Fax: +86 754 8890 0437.
E-mail address: andytylau@stu.edu.cn (A.T.Y. Lau).
[1] These authors contributed equally to this work.

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

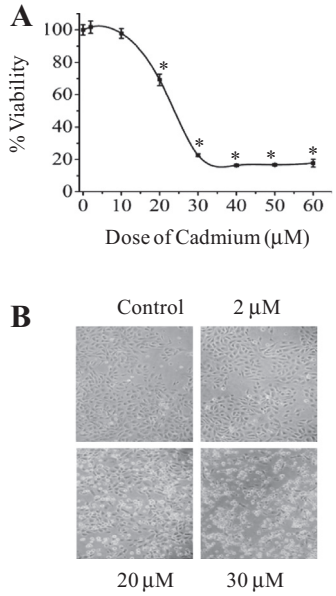

Fig. 1. Dose-dependent cytotoxicity of Cd on BEAS-2B cells. (A) Cells were plated in 96-well plates at 2×10^4/well and incubated overnight. On the next day, cells were dosed with $CdCl_2$. After 36 h, cell viability was measured by NBB staining assay. The percentage of viability was plotted as 100% for control (no treatment of Cd). Results are expressed as mean ± S.D. of triplicate samples and reproducibility was confirmed in three separate experiments. *A significant difference ($P < 0.05$) as compared with control. (B) Cell morphology of BEAS-2B cells treated with various dosage of $CdCl_2$ for 36 h under light microscope.

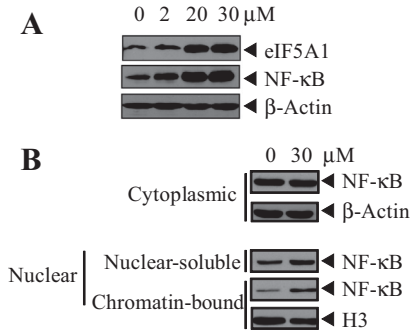

Fig. 2. $CdCl_2$ treatment induces the upregulation of eIF5A1 and NF-κB p65 in BEAS-2B cells. (A) BEAS-2B cells were treated with $CdCl_2$ for 36 h; cells were lysed; and protein extracts were subjected to Western blot analysis using antibodies against eIF5A1 and NF-κB p65. (B) BEAS-2B cells were treated with 30 μM $CdCl_2$ for 24 h, individual subcellular fractions (cytoplasmic, nuclear-soluble, and chromatin-bound) were isolated and subjected to Western blot analysis using NF-κB p65 antibody. The same blot was stripped and reprobed with the monoclonal β-actin or histone H3 antibody to monitor the loading difference. Data are representative of three independent experiments.

subjected to trypsin digestion, MALDI–TOF mass spectra measurement and database searching (data not shown). Supplementary Table 2 summarizes the identified proteins and their alterations between normal control and 30 μM Cd-treated cells. The upregulated proteins are mostly related to anti-oxidative/stress response proteins, such as GSTP1, HSPA6 and HSP90B1. Interestingly, we also identified eukaryotic translation initiation factor 5A-1 (eIF5A1) as one of the Cd-responsive proteins. Although the cellular function of eIF5A1 is reported to be related to protein biosynthesis, recent findings suggested that it is also a pro-apoptotic protein that participated in TNFα-mediated apoptosis of lamina cribrosa cells and induced apoptosis in A549 lung cancer cells which is not dependent on p53 activity [13,14].

3.4. Involvement of NF-κB activation in BEAS-2B cells upon Cd exposure

As mentioned above, although we observed the upregulation of eIF5A upon Cd treatment in BEAS-2B cells, however, the p53 level was not upregulated but rather downregulated in a time- and dose-dependent manner, suggesting that eIF5A1 expression is not likely regulated by p53. This prompted us to study other transcription factors that might be involved. Surprisingly, we determined that the redox-sensitive transcription factor, NF-κB, is also upregulated following the same pattern as of eIF5A1 upon Cd treatment (Fig. 2A), suggesting that NF-κB activation might be linked to eIF5A1 induction.

3.5. Increase of NF-κB nuclear translocation and chromatin-binding upon Cd exposure in BEAS-2B cells

Nuclear translocation of NF-κB is a prerequisite for its specific binding to the responsive elements located at the promoter region of NF-κB-target genes. Therefore, determining the subcellular localization of NF-κB can delineate its activity inside cells. We fractionated individual subcellular components of Cd-treated cells and it was found that the levels of nuclear NF-κB, whether in the nuclear-soluble and chromatin-bound fractions, were increased as compared with untreated control. This indicates that there was an enhanced NF-κB nuclear translocation as well as chromatin-binding in Cd-treated BEAS-2B cells, and suggests NF-κB might likely be involved in modulating *eIF5A1* gene expression.

3.6. The effects of eIF5A1 silencing on Cd sensitivity

From our results, we suggest that if increased eIF5A1 was involved in Cd-induced cell death, then removal of eIF5A1 should attenuated the Cd cytotoxicity. For this reason, we resolved to use loss-of-function method to silence the eIF5A1 in BEAS-2B cells by siRNA technique. To test the effect of silencing of the *eIF5A1* gene on Cd cytotoxicity, BEAS-2B cells were transfected with 100 or 200 nM siRNA and grown for 36 h. The samples were processed for Western blot analysis to determine the expression of eIF5A1. As expected, transfection of BEAS-2B cells with 100 or 200 nM siRNA resulted in almost complete reduction of *eIF5A1* gene expression (Fig. 3A). More importantly, the silencing of eIF5A1 before the addition of Cd sustained the cell viability of BEAS-2B cells compared with Cd-treated control cells (Fig. 3B and C). These results clearly showed that silencing of eIF5A1 significantly protected the Cd-induced cytotoxicity in BEAS-2B cells. To sum up, based on the results obtained from silencing of eIF5A1 in BEAS-2B cells by siRNA, we conclude that the elevated expression of eIF5A1 upon Cd treatment is crucial in promoting cell death.

3.7. Enhanced NF-κB binding on eIF5A1 promoter region of Cd-treated cells

Our results thus so far suggested the involvement of NF-κB activation and elevated eIF5A1 expression in Cd-treated BEAS-2B cells. 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. To this end, we first analyzed the whole *eIF5A1* gene and upstream 1500 bp sequence. Fig. 4A shows the schematic structure of the human *eIF5A1* gene and proximal promoter region. By bioinformatic analysis, six potential NF-κB binding sites (four on the proximal promoter region and two on the first intron) on *eIF5A1* gene can be found. We therefore designed six primer pairs (Supplementary

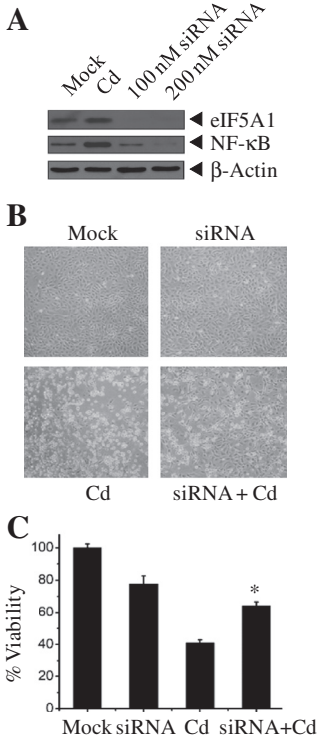

Fig. 3. eIF5A1 silencing in BEAS-2B cells. BEAS-2B cells were transfected with eIF5A1 siRNA duplexes. (A) Cells were harvested at 36 h for Western blot analysis. Cells treated with Cd served as positive control of eIF5A1 and NF-κB p65 induction for relative comparison. (B) After eIF5A1 siRNA transfection (100 nM siRNA), cells were treated with $CdCl_2$ for 36 h, shown are cell morphology under light microscope. (C) Corresponding NBB staining assay of (B) for the determination of viability. The percentage of viability was plotted as 100% for control (no treatment of Cd). Columns, means of triplicate samples and reproducibility was confirmed in three separate experiments; bars, S.D. *, $P < 0.05$ versus Cd-treated cells only.

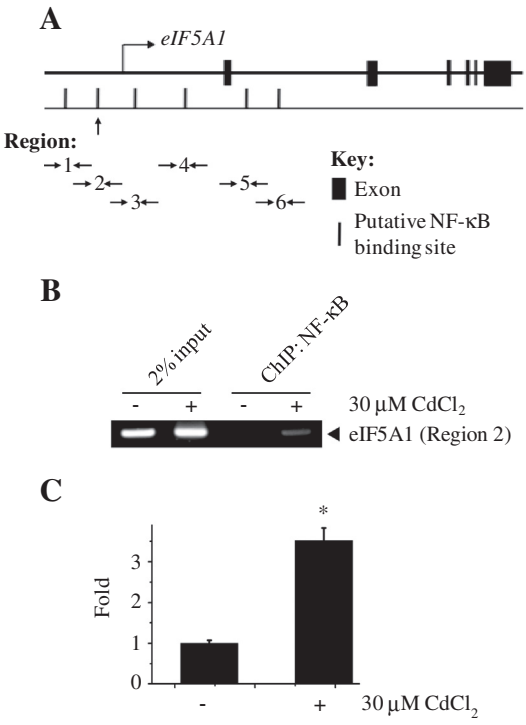

Fig. 4. Enhanced NF-κB binding on *eIF5A1* promoter region of Cd-treated cells. (A) Schematic structure of the human *eIF5A1* gene. NF-κB binds only to putative binding site in region 2 (pointed arrow) as determined by ChIP assay. (B) Chromatin immunoprecipitation assay of *eIF5A1* promoter. Equal amounts of cross-linked chromatin were incubated with NF-κB p65 antibody. Following DNA purification, samples were analyzed by PCR using primers specific to region 2 of *eIF5A1* promoter. PCR using total input chromatin from sham-exposed or Cd-treated cells as templates serve as positive controls for the primers. Results similar to those depicted here were obtained in at least two other independent sets of ChIP experiments. (C) Corresponding normalized values of bands in (B) were presented as a fold increase (set at 1 for sham-exposed cells). The data are shown as the mean ± S.D., and the asterisk indicates a significant difference ($P < 0.05$).

Table 1) that each cover these putative binding sites for ChIP-PCR assay to determine whether there is enhanced NF-κB binding upon Cd treatment (Fig. 4A). Notably, our ChIP assay results revealed that among the six putative sites, only site 2 showed the presence of and elevated NF-κB binding as evidenced by a 3.5-fold enrichment of PCR products (Fig. 4B and C); while the other putative sites, i.e. sites 1, 3, 4, 5, and 6 all failed to show any PCR product after the ChIP assay (data not shown). This suggests that site 2, which is closed to the transcription start site, is likely the location where nuclear translocated NF-κB binds and subsequently activates eIF5A1 transcription. Based on our results, we suggest for the first time a regulatory mechanism for the pro-apoptotic protein eIF5A1 in which its level is possibly modulated by NF-κB.

4. Discussion

In this study, we studied the transient effect of $CdCl_2$ in human bronchial epithelial cells (BEAS-2B). By the use of proteomic approach, we were able to identify the responsive proteins that are altered by Cd exposure. Among these responsive proteins that we identified, the function of eIF5A1 was further evaluated. More importantly, we showed that siRNA-specific knockdown of eIF5A1 in BEAS-2B cells attenuated the Cd cytotoxicity, indicating the pro-apoptotic role of eIF5A1 that it plays during Cd-induced cell death. eIF5A1 is a small protein (16.8 kD) that is ubiquitously-expressed and highly-conserved among eukaryotes and is the only known protein that is post-translationally modified on a conserved lysine residue (K50) by two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), which transfer a butylamine group from spermidine to a conserved lysine residue to produce the amino acid, hypusine [15,16]. Although eIF5A1 has been shown as an mRNA-binding protein involved in translation elongation, depletion of eIF5A1 in yeast caused only a small reduction in rate of protein synthesis [17,18]. Recently, eIF5A1 was demonstrated as a specific factor in promoting poly-proline peptide bond formation [19]. Previous studies by overexpression experiments indirectly showed that eIF5A1 is involved in cell apoptosis but the function of eIF5A1 in apoptosis is still obscure. Introduction of the wild-type eIF5A1 or its mutant which cannot be hypusinated at K50 were both effective in promoting apoptosis in A549 cells, suggesting that hypusination is not required for eIF5A1 to exert its apoptotic function [14].

In our study, by examining the endogenous protein levels in BEAS-2B cells, we demonstrated that Cd can potently induce eIF5A1 in this normal lung cell model while p53 is severely downregulated. It has been shown that eIF5A1 regulation is p53-dependent in $p53^{+/+}$ HCT116 colon cancer cells [20]. However, our studying human lung cell model behaved differently which is in contrary to other reports that eIF5A expression is regulated by p53. As a matter of fact, there are also reports showing the downregulation of p53 protein levels upon Cd treatment [21–23]. Our results are in agreement with others which demonstrated the downregulation of p53 by Cd treatment in lung fibroblast WI 38 and rat testes [21–23], and induction of apoptosis in response to eIF5A1 in A549 lung cancer cells which is not dependent on p53 activity [14].

It is conceivable that the redox-sensitive transcription factor is activated during Cd-induced cytotoxicity as Cd is well-known for

its ability to generate ROS inside cells, which alters the cellular redox-status [6–8]. As a matter of fact, as low as 2 μM $CdCl_2$ treatment, we can see that there were already some observable elevations of NF-κB and eIF5A1 levels (Fig. 2A), yet, we do not observe a subtle cytotoxicity to BEAS-2B cells at this low dosage of Cd treatment (Fig. 1), suggesting that although low and high levels of Cd can activate this pathway, the levels of eIF5A1/activated NF-κB induced are crucial in dictating the cell fate that ultimately lead to survival or cell death. Nevertheless, further study is required to address these issues.

Although the dosage range of Cd that we used is capable of inducing cell death in BEAS-2B. However, not 100% of these Cd-exposed cells are killed. As a result, a small fraction of cells might actually recover along with suppressed p53 expression. The consequence of p53 under-expression is significant as p53 is the principal guardian in maintaining genome stability such that reduced or diminished p53 could then lead to neoplastic cell survival as well as subsequent clonal expansion and then progression of tumor development [7,21].

In summary, in this acute Cd exposure scenario in BEAS-2B cells, our findings report for the first time that the pro-apoptotic protein eIF5A1 is involved in Cd-induced cell death in human lung cells and possibly modulated by the NF-κB pathway. This is supported by the fact that there is enhanced NF-κB binding on *eIF5A1* promoter region of Cd-treated BEAS-2B cells and introduction of eIF5A1 siRNA before Cd treatment effectively prevented cell death. Moreover, we show also the potential of Cd to severely suppressing p53 expression which could likely lead to genomic instability and the promotion of carcinogenesis in Cd tolerant cells. Further studies are underway to examine the effect of long-term Cd exposure on these cells and will hopefully provide more insights into this area in the future.

Acknowledgments

This work was supported by National Natural Science Foundation of China Grants 31170785 and 81101785 (Andy T.Y. Lau), Fund for University Talents of Guangdong Province (Andy T.Y. Lau), and Guangdong Natural Science Foundation of China Grant S2012030006289. We would like to thank members of the **L**au **A**nd **X**u laboratory for critical reading of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.146.

References

[1] M.P. Waalkes, T.P. Coogan, R.A. Barter, Toxicological principles of metal carcinogenesis with special emphasis on cadmium, Crit. Rev. Toxicol. 22 (1992) 175–201.
[2] C.T. De Rosa, B.L. Johnson, M. Fay, H. Hansen, M.M. Mumtaz, Public health implications of hazardous waste sites: findings, assessment and research, Food Chem. Toxicol. 34 (1996) 1131–1138.
[3] International Agency for Research on Cancer, IARC monographs on the evaluation of the carcinogenic risks to humans. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry, IARC 58 (1993) 119–238.
[4] L. Magos, Epidemiological and experimental aspects of metal carcinogenesis: physicochemical properties, kinetics, and the active species, Environ. Health Perspect. 95 (1991) 157–189.
[5] L. Järup, T. Bellander, C. Hogstedt, G. Spång, Mortality and cancer incidence in Swedish battery workers exposed to cadmium and nickel, Occup. Environ. Med. 55 (1998) 755–759.
[6] C.F. Yang, H.M. Shen, Y. Shen, Z.X. Zhuang, C.N. Ong, Cadmium-induced oxidative cellular damage in human fetal lung fibroblasts (MRC-5 cells), Environ. Health Perspect. 105 (1997) 712–716.
[7] A. Hartwig, Mechanisms in cadmium-induced carcinogenicity: recent insights, Biometals 23 (2010) 951–960.
[8] A. Hartwig, Cadmium and cancer, Met. Ions Life Sci. 11 (2013) 491–507.
[9] B.A. Hart, C.H. Lee, G.S. Shukla, A. Shukla, M. Osier, J.D. Eneman, J.F. Chiu, Characterization of cadmium-induced apoptosis in rat lung epithelial cells: evidence for the participation of oxidant stress, Toxicology 133 (1999) 43–58.
[10] Y.M. Xu, Y. Zhou, D.J. Chen, D.Y. Huang, J.F. Chiu, A.T.Y. Lau, Proteomic analysis of cadmium exposure in cultured lung epithelial cells: evidence for oxidative stress-induced cytotoxicity, Toxicol. Res. 2 (2013) 280–287.
[11] A.T.Y. Lau, M. Li, R. Xie, Q.Y. He, J.F. Chiu, Opposed arsenite-induced signaling pathways promote cell proliferation or apoptosis in cultured lung cells, Carcinogenesis 25 (2004) 21–28.
[12] E. Portales-Casamar, S. Thongjuea, A.T. Kwon, D. Arenillas, X. Zhao, E. Valen, D. Yusuf, B. Lenhard, W.W. Wasserman, A. Sandelin, JASPAR 2010: the greatly expanded open-access database of transcription factor binding profiles, Nucleic Acids Res. 38 (2010) D105–D110.
[13] C.A. Taylor, M. Senchyna, J. Flanagan, E.M. Joyce, D.O. Cliche, A.N. Boone, S. Culp-Stewart, J.E. Thompson, Role of eIF5A in TNF-alpha-mediated apoptosis of lamina cribrosa cells, Invest. Ophthalmol. Vis. Sci. 45 (2004) 3568–3576.
[14] C.A. Taylor, Q. Zheng, Z. Liu, J.E. Thompson, Role of p38 and JNK MAPK signaling pathways and tumor suppressor p53 on induction of apoptosis in response to Ad-eIF5A1 in A549 lung cancer cells, Mol. Cancer 12 (2013) 35.
[15] E.C. Wolff, K.R. Kang, Y.S. Kim, M.H. Park, Posttranslational synthesis of hypusine: evolutionary progression and specificity of the hypusine modification, Amino Acids 33 (2007) 341–350.
[16] M.H. Park, K. Nishimura, C.F. Zanelli, S.R. Valentini, Functional significance of eIF5A and its hypusine modification in eukaryotes, Amino Acids 38 (2010) 491–500.
[17] A.P. Gregio, V.P. Cano, J.S. Avaca, S.R. Valentini, C.F. Zanelli, EIF5A has a function in the elongation step of translation in yeast, Biochem. Biophys. Res. Commun. 380 (2009) 785–790.
[18] H.A. Kang, J.W. Hershey, Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*, J. Biol. Chem. 269 (1994) 3934–3940.
[19] E. Gutierrez, B.S. Shin, C.J. Woolstenhulme, J.R. Kim, P. Saini, A.R. Buskirk, T.E. Dever, EIF5A promotes translation of polyproline motifs, Mol. Cell 51 (2013) 35–45.
[20] R. Rahman-Roblick, U.J. Roblick, U. Hellman, P. Conrotto, T. Liu, S. Becker, D. Hirschberg, H. Jörnvall, G. Auer, K.G. Wiman, P53 targets identified by protein expression profiling, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 5401–5406.
[21] G. Xu, G. Zhou, T. Jin, T. Zhou, S. Hammarström, A. Bergh, G. Nordberg, Apoptosis and p53 gene expression in male reproductive tissues of cadmium exposed rats, Biometals 12 (1999) 131–139.
[22] S.H. Oh, B.H. Lee, S.C. Lim, Cadmium induces apoptotic cell death in WI 38 cells via caspase-dependent Bid cleavage and calpain-mediated mitochondrial Bax cleavage by Bcl-2-independent pathway, Biochem. Pharmacol. 68 (2004) 1845–1855.
[23] G.Y. Li, M. Kim, J.H. Kim, M.O. Lee, J.H. Chung, B.H. Lee, Gene expression profiling in human lung fibroblast following cadmium exposure, Food Chem. Toxicol. 46 (2008) 1131–1137.