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eIF3 regulate migration, invasion and apoptosis in cadmium transformed 16HBE cells and is a novel biomarker of cadmium exposure rat model and workers

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Running title: *eIF3* in cadmium toxicity

Abstract

Translation (eukaryotic) initiation factor 3 (*eIF3* or *TIF3*) has been found to be a proto-oncogene in cadmium (Cd) response both in vitro and vivo, but whether *eIF3* may serve as a biomarker of Cd exposure is still unclear. This study aimed to investigate whether *eIF3* could serve as a as a novel biomarker of Cd toxicity in cells, animals and workers, and regulate the apoptosis, migration and invasion in human bronchial epithelial cells (16HBE cells) transformation with cadmium chloride ($CdCl_2$). In $CdCl_2$ transformed 16HBE cells, *eIF3* expression increased gradually， sequencing did not identify mutation and methylation of *eIF3*. In 16HBE cells with *eIF3* silencing by siRNA and $CdCl_2$ treated 16HBE cells of 15^{th} and 35^{th} generations, the apoptosis, migration and invasion were significantly inhibited, and the expressions of relevant genes were also altered (P<0.05). In $CdCl_2$ treated rats, *eIF3* mRNA expression increased to different extents in the blood, liver, kidney, heart and lung, and this increase was dependent on the Cd concentration (P<0.05). The *eIF3* mRNA expression was related to the mRNA expressions of *AKT, BAX, BCL2, E-CADHERIN*， *CASPASE3, EGFR, FOXC2, STAT3, TGF-β1* and *VIMENTIN* (P<0.05). In 181 workers with Cd exposure, the *eIF3* mRNA expression was positively related to the blood Cd, urine Cd and *β2*-microglobulin content (P<0.05). This study showed abnormally expressed *eIF3* may regulate the apoptosis, migration and invasion of 16HBE cells with Cd toxicity. This suggests that *eIF3* may become

a novel and valuable biomarker of Cd toxicity and Cd-induced effects, and may regulate apoptosis, migration and invasion of 16HBE cells. Thus, to detect *eIF3* expression is important for the monitoring of Cd toxicity in humans.

Key words: Cadmium toxicity, human bronchial epithelial cells, *eIF3*, biomarker

Introduction

Cadmium (Cd) and its compounds are considered as harmful pollutants worldwide[1-3]. Cd has a long biological half-time (19–30 years), and can accumulate and be present in multiple organs for a long time. Cd is toxic to organs and can lead to a number of diseases including liver and kidney injury, respiratory diseases, neurological disorders, skeletal system damage and reproductive system disorder [4-6]. Cd is the 7th priority toxicant according to the Agency for Toxic Substances and Disease Registry (ATSDR) of the United States[7]. Based on the epidemiological and laboratory findings, Cd has been reported to cause cancer in many organs including the kidney, liver, lung, prostate, pancreas, bladder and breast[8-12,]. In 1993, Cd and its compounds were named as Group 1 carcinogen by the International Agency for Research on Cancer (IARC)[13,14]. Therefore, a sensitive and specific biomarker for Cd exposure is beneficial. Although some of the molecules involved in Cd tolerance have been identified, the potential mechanisms involved are still largely unknown.

There is evidence showing that Cd is a potent inducer of gene mutation and may cause uncontrollable gene expression. Some toxicological properties of Cd determine that Cd may alter the expressions of key functional genes in target organs, which has been focused in cells and animals exposed to Cd[15]. In several studies on the Cd induced carcinogenesis, the expressions of some translation-related genes are abnormal. Translation factors involved in the

protein expression in eukaryotic cells include Translation initiation factor, translation elongation factor and translation termination factor. These factors play important roles in the growth, proliferation and malignant transformation of normal cells[16,17]. *eIF3* is the largest translation initiation factor in eukaryotic cells, participates in several steps of translation initiation and plays a central role in the translation initiation[18,19].

Studies have shown that *eIF3* may form stable complexes with 40S ribosomal subunit, which may prevent against early binding to 60S ribosomal subunit. *eIF3* is indispensable for the stable binding of *eIF2*-GTP-MettRNA triple complex to 40S subunit. *eIF3* binds to the *eIF4G* subunit of cap-binding protein complex (eIF4E) when the 40S subunit is brought to Mrna[20, 21]. In addition, *eIF3* may interact with *eIF4B, eIF5* and *eIF1*, which suggests that *eIF3* play a central role in the translation initiation via interacting with different initiation factors[18,19]. Studies also reveal that abnormal *eIF3* high expression may cause the malignant transformation of cells, and *eIF3* is highly expressed in transformed cells, breast cancer cells and prostate cancer cells[22,23]. The stable expression of modified *eIF3e*(p48) gene in NIH3T3 cells may cause malignant transformation[24]. Joseph and Lei et al[25,26] investigated the gene expression profiles in $CdCl_2$ transformed BALB/c-3T3 cells. They found that *eIF3* (GenBank accession number AF271072) in rats was highly expressed in $CdCl_2$ transformed cells and *eIF3* was identified as a proto-oncogene of Cd response. However, in Cd toxicity, what the regulatory capability of *eIF3* is, what the

mechanism underlying the abnormal expression of *eIF3* is and whether *eIF3* may serve as a biomarker for Cd exposure are still unclear.

We previously established a model of morphological cell transformation with cadmium chloride ($CdCl_2$) in human bronchial epithelial cells (16HBE)[27] and a Cd exposure model in rats [28]. As described previously, to establish the cells transformed with $CdCl_2$, 16HBE cells were malignantly transformed by continuous treatment of $CdCl_2$ and formed tumors in null mice, and cells isolated from the xenograft tumors were established to cell. Reproducibly, 16HBE cells treated with $CdCl_2$ for 35 passages can form robust colonies in soft agar and initiate xenograft tumors in nude mice indicated the fully malignant transformation. To establish the Cd exposure model in rats, Specific-pathogen-free (SPF) Sprague-Dawley (SD) rats were chronically exposed to Cd by intra-peritoneal injection of $CdCl_2$. Cd treatment was performed five times weekly for 14 weeks. These models are helpful to examine the molecular events occurring during Cd toxicity and carcinogenesis. This study aimed to investigate the change in *eIF3* expression and its potential mechanism and to explore the regulatory effects of *eIF3* on the apoptosis, proliferation, migration and invasion of cells. Moreover, we validated *eIF3* as a novel biomarker of Cd toxicity in cells, animals and workers with Cd exposure.

Materials and methods

Cell culture and treatments

16HBE cells were morphologically transformed using $CdCl_2$, as previously described[27]. Un-transformed 16HBE cells (control group); Cd-transformed cells of the 5th (5 μmol/L Cd for 2 weeks), 15th (5 μmol/L Cd for 6 weeks) and 35th (5 μmol/L Cd for 14 weeks) passages were maintained in RPMI-1640 containing L-glutamine, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Life Technologies) at 37°C in a 5% CO_2 humidified atmosphere. The cells were passaged twice weekly and cells in logarithmic growth phase (2-5×10^5 cells/mL) were harvested for following experiments.

Animals and Cadmium Exposure

Specific-pathogen-free (SPF) Sprague-Dawley (SD) rats (90 ± 10 g) were purchased from Guangdong Medical Laboratory Animal Center (Licence No: SCXK 2008-0002, Guangdong, China) and maintained under a pathogen-free condition in Laboratory Animal Center of Guangzhou Army General Hospital [Licence No: SYXK (Military) 2007-33, 2008C1230034834, Guangdong, China]. Ninety-six SD rats (half male and half female) were randomly divided into 4 groups. Rats were chronically exposed to Cd by intra-peritoneal injection of $CdCl_2$ (Sigma, St. Louis, MO, USA) in normal saline at different concentrations (high-dose: 1.225 mg/kg; mid-dose: 0.612 mg/kg and low-dose: 0.306 mg/kg). Rats in control group were intra-peritoneally injected with 0.5 mL of normal saline. Cd treatment was performed five times weekly. After 14

weeks, 24-h urine samples were collected. On the second day, rats were anesthetized and blood was collected from the heart and stored at 4℃. The liver, kidney, heart and lung were harvested and stored in liquid nitrogen. The animal handling and experimental procedures were approved by the Animal Experimental Ethics Committee of Guangzhou Army General Hospital (Guangzhou, China)[28].

Study population

A total of 181 workers were recruited from the Cd refinery factory with the assistance of Center for Disease Control and Prevention, Institute for Health Supervision in Shenzhen, P.R. China. The workers included production workers, machine maintenance workers, product development personnel, management personnel and other personnel engaged in cleaning, service, security, and so on. Detailed information including the age, marital status, smoking status, alcohol consumption, professional and medical history was collected from each subject and evaluated by well-trained interviewers. In addition, the workers were asked to receive a comprehensive physical examination. The physical examination included detection of blood pressure and pulse rate, examination of the throat and pharynx, detection of lung function, electrocardiogram, liver and kidney ultrasonography, cardiopulmonary X-ray, and detection of blood cells, serum alanine aminotransferase (ALT), urinary Cd and creatinine (Cr). In this study, subjects who could not provide reliable information on the smoking history, had a smoking history or had a history of kidney or liver diseases were

excluded. Finally, 181 non-smoking subjects (109 males and 72 females) aged 23-50 years were included for analysis.

Quantitative real-time PCR

Total RNA was isolated using the TRIzol reagent. The RNA purity，integrity，and concentration were assessed by agarose gel electrophoresis and NanoDrop ND-1000(Thermo Fisher Scientific Inc.USA). Reverse transcription was performed using a TITANIUM real-time PCR (RT-PCR) kit (Clontech, Mountain View, CA) according to the manufacturer's instructions. The gene expression was quantified using a fluorescence-based RT-PCR. Data were normalized to β-actin levels according to manufacturer's instructions (Bio-Rad Laboratories). The sequences of primers used for RT-PCR are shown in Table 1.

RNA interference

To inhibit *eIF3*, 50 nM of siRNA (siRNA *eIF3-1,* siRNA *eIF3-2*, siRNA *eIF3-3*)，Shanghai Genepharma, China) were transfected into untreated 16HBE cells，Cd-transformed cells of the 15th passage and Cd-transformed cells of the 35th passage using Lipofectamine 2000 reagent according to the manufacturer's instructions. Cells transfected with scramble-control siRNA (negative control) were used as controls. Cells were harvested 72 h after transfection. Compared with controls, three siRNA *eIF3* successfully decreased the expression of *eIF3*. The sequences of *eIF3* siRNA and scramble control siRNA are listed in Table

1.

Cell migration and invasion assay

Cell migration was assessed using Control Cell Culture Inserts in two 24-well plates of 8 μm (BD Biosciences) according to the manufacturer's instructions. Briefly, 200 μl of serum-free medium containing 2×10^5 cells from each subgroup were add to the upper chamber, and 0.6 ml of 20% FBS-containing medium was then added to the lower chamber as a chemoattractant. Cells were incubated for 16 h at 37°C in 5% CO_2.

Matrigel Invasion Chambers in two 24-well plates of 8 μm (BD Bioscience) were used for the invasion assay according to the manufacturer's instructions. Briefly, 200 μl of serum-free medium containing 1×10^5 cells from each subgroup were add to the upper chamber and 0.6 ml of 20% FBS-containing medium was then added to the lower chamber as a chemoattractant. Cells were incubated for 40 h at 37℃ in 5% CO_2.

After the incubation, cells on the upper surface of the membrane were removed with cotton swabs. Cells migrating to the bottom of the membrane were fixed and stained with 0.1% Crystal Violet. Cells on the bottom of the membrane were counted at five different microscopic fields and the average was calculated. All above experiments were replicated three times.

Cytometric analysis of apoptosis cells

To explore the effect of *eIF3* on Cd-transformed cells, detection of apoptosis in 16HBE cell, Cd-transformed cells of the $15^{th}$and 35^{th} passages cells were

carried out after transfected with siRNA *eIF3* only for 72 h. Apoptosis cells were analyzed using a flow cytometer (CYTOMICS FC 500, Beckman Coulter) after incubating with reagent containing Annexin V-FITC and Propidium Iodide (BD Bioscience, San Jose, CA) for 15 min in darkness at room temperature. Each study was repeated four times.

Sequencing of *eIF3*

DNA extraction kit (QIAGEN) was used to extract total DNA from un-transformed 16HBE cells (control group). Cd-transformed cells of the 5th passage, 15th passage and 35th cells passages Seven paired primers were designed on the basis of sequences obtained from http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi and processed for PCR. The maximum and minimum lengths of 7 segments were 1266 bp and 350 bp, respectively and these segments covered the exons and promoter with 1 kb of *eIF3* gene. PCR products were subjected to purification with Shrimp alkaline enzyme (SAP, Promega) and exonuclease I (EXO I, Epicentre) and then processed with BigDye3.1 kit (ABI). The sequencing reactants were purified with ethanol and the sequences of exons and promoter of *eIF3* gene were determined with ABI3130XL sequencer. Results were analyzed and compared with Polyphred software.

Detection of *eIF3* methylation

Total DNA was extracted from un-transformed 16HBE cells (control group); Cd-transformed cells of the 5th passage, 15th passage and 35th cells passages.

Then, 1 μl of DNA was subjected to 1% agarose electrophoresis for the detection of concentration and quality. EZ DNA Methylation-Gold kit (ZYMO RESEARCH) was used for methylation treament, and the methylated CpG island, full exons and promoter were sequenced. A total of 13 segments (11 at exons [2002..2076, 2170..2262, 3666..3753, 4042..4107, 5803..5952, 6035..6162, 8131..8241, 8359..8448, 8767..8840, 10559..10651, 10747..>11235] and 2 at promoter) were sequenced.

Network Diagram of genes regulated by *eIF3*

Data were collected from mRNA microarray assay, microRNA microarray assay and lncRNA microarray assay and screening was done according to the fold change. The $P < 0.05$ and fold change of ≥ 2 were used thresholds for screening of differentially expressed mRNA, microRNA and lncRNA. Cytoscope software was employed to delineate network diagram of *eIF3*-mRNA co-expression.

Network diagram of *eIF3* regulated by ceRNA

When the miRNA was negatively related to mRNA and miRNA-mRNA expression (pearson correlation coefficient <-0.8), the predicted network between miRNA and *eIF3* targets was established as follows: the target gene of known miRNA was predicted from intersection of Mireap, miRanda, targetscan, MiRanda (http: //www.microrna.org/) with default parameters, and the input was mature miRNA sequence and 3'UTR of gene. TargetScan (http://www.targetscan.org/) with default parameters, the input is 7mer sed of

mature RNA and 3'UTR of gene. Mireap (http://sourceforge.net/projects/mireap) with default parameters, the input was mature miRNA sequence and 3'UTR of gene. Then, the regulatory relationship between miRNA and lncRNA was established. The regulatory networks of miRNA-mRNA and miRNA-lncRNA obtained on the basis of above procedures were employed to delineate the regulatory network of lncRNA-miRNA-mRNA by using cytoscope software.

Cadmium determination and functional and pathological examinations of organs in cadmium-exposed rats

The cadmium level was determined using the cadmium standard solution (BZ/WJ/GB101/2009-1, Guangdong Occupational Health Inspection Center, Guangdong, China) by atomic absorption spectrometry (ZEENIT700, Analytik Jena, Jena, German). The concentration of urine cadmium was normalized by urinary creatinine (*Cr*). Tissue samples were fixed in 10% formalin and the pathological features were examined following Hematoxylin and Eosin (HE) staining. Serum alanine aminotransferase (*ALT*) and aspartate aminotransferase (*AST*) were used as biochemical markers of liver function. Blood urea nitrogen (*BUN*), serum creatinine (*sCr*) and 24-h urine protein (24-h Pro) were used to evaluate the renal function. *ALT, AST, BUN, SCR* and 24-h Pro were measured using corresponding kits according to the manufacturer's instructions with an automatic biochemistry analyzer (Hitachi 7600- 020/7170A: Tokyo, Japan). All animal experiments were performed in accordance with the principals of the

Declaration of Helsinki. All experimental protocols were approved by Research Ethic Committee of Guangzhou Medical University.

Collection and treatment of biological samples from Cadmium-exposed workers

Venous blood was collected after fasting for 10-12 h and transferred into anticoagulant and metal-free tube for blood cadmium (BCd) detection, blood routine examination, blood biochemical examination (*ALT, AST, Cr* and *BUN*), and detection of blood *eIF3* and its target genes. BCd concentrations were measured by atomic absorption spectrometry (ZEENIT700; Analytik Jena, Jena, Germany). Blood biochemistry was done with an automatic biochemical analyzer (HITACHI7600-020/7170A; Hitachi, Tokyo, Japan). The expression of *eIF3* and its target genes was measured by quantitative real-time PCR.

Urine samples were collected from all participants and transferred into a metal-free polyethylene bottle as per the guidelines of clinical chemistry division of International Union of Pure and Applied Chemistry. These samples were diluted with equal volume of 0.3 mol/L HNO_3 and stored at 4°C untill further analysis. Urine Cd concentration was measured by atomic absorption spectrometry (ZEENIT700; Analytik Jena, Jena, Germany). Cd standard curve was linear up to 25 μg/L and the detection limit was 0.33 μg/L. The internal standard of Cd was added to urine and analyzed, and a recovery rate of 98.2% was found. Urinary beta2-microglobulin (Uβ2-MG) levels were measured by radio-immunoassay (RIA) (Pharmacia β2-micro RIA, Pharmacia Diagnostics

AB, Sweden).

All the human experiements were performed in accordance with the principals of the Declaration of Helsinki. All experimental protocols were approved by Research Ethic Committee of Guangzhou Medical University. The personal information of samples involved in the study was not opened.

Statistical analysis

All the data are represented as rate (%) or mean ± standard deviation (SD; $\bar{x} \pm$ s) of three or more independent experiments. Comparisons were done using the chi square test for rate (%) from several independent experiments and Student's t-test or analysis of variance (ANOVA) followed by Dunnett's test for mean ±SD. The correlation of two groups was tested by Pearson or Spearman's correlation analysis. Statistical analysis was performed with SPSS version 13.0 software. A value of $P<0.05$ was considered statistically significant.

Results

Abnormally high *eIF3* expression in $CdCl_2$ transformed 16HBE cells

Real time PCR was performed to detect the *eIF3* mRNA expression in $CdCl_2$ transformed 16HBE cells at different stages. Results showed the *eIF3* mRNA expression increased over time in $CdCl_2$ transformed 16HBE cells. The *eIF3* mRNA expression in 16HBE cells of 5^{th} passage, 16HBE cells of 15^{th} passage, and 16HBE cells of 35^{th} passage was 3.6, 3.0 and 9.1 times that in control group of the same passage numbers ($P<0.05$). These suggest that there is abnormally high *eIF3* expression in $CdCl_2$ transformed 16HBE cells.

Regulatory effects of *eIF3* on the invasion and migration of $CdCl_2$ transformed 16HBE cells

The invasion and migration of $CdCl_2$ transformed 16HBE cells increased gradually. While the invasion and migration reduced markedly in 16HBE cells, 16HBE cells of 15^{th} passage, and 16HBE cells of 35^{th} passage after *eIF3* silencing, accompanied by changes in the mRNA expressions of genes related to cell migration and invasion. Of these genes, *EGFR, STAT3, FOX2* and *VIMENTIN* showed markedly reduced mRNA expression, but *E-CADHERIN* and *AKT* presented with significantly increased mRNA expression ($P<0.05$). Results showed the invasion and migration of $CdCl_2$ transformed 16HBE cells were abnormal and *eIF3* may exert regulatory effects on the migration and invasion of these cells (Figure 1A, B, C).

Regulatory effects of *eIF3* on the apoptosis of $CdCl_2$ transformed 16HBE

cells

Flow cytometry was performed to detect the apoptosis of 16HBE cells before and after *eIF3* silencing. When compared with control group, the number of apoptotic cells reduced markedly in 16HBE cells, 16HBE cells of 15th passage and 16HBE cells of 35th passage after *eIF3* silencing, accompanied by changes in the mRNA expressions of apoptosis related genes. Of these genes, BAX and caspase-3 showed markedly reduced mRNA expression, and *BCL-2* had significantly increased mRNA expression ($P<0.05$). These results suggest that *eIF3* is able to regulate the apoptosis of $CdCl_2$ transformed 16HBE cells (Figure 2A, B, C).

Sequencing of exons and promoter of *eIF3* in $CdCl_2$ transformed 16HBE cells

The whole exons and promoter were sequenced in un-transformed 16HBE cells (control group); Cd-transformed cells of the 5th passage, 15th passage and 35th cells passages (11 segments in exons and 2 segments in promoter). One known SNP was identified, and point mutation was not found. These suggest that the abnormal *eIF3* expression is not related to the *eIF3* gene mutation in $CdCl_2$ transformed 16HBE cells.

Squencing for *eIF3* methylation in $CdCl_2$ transformed 16HBE cells

The predicted CpG island was subjected to expansion and sequencing after pre-treatment in un-transformed 16HBE cells (control group). Cd-transformed cells of the 5th passage, 15th passage and 35th cells passages (2 pairs of primers

used for expansion of 2 segments [250 bp and 202 bp]). Results showed there was no gene methylation in 4 types of cells. This suggests that abnormal *eIF3* expression is not related to DNA methylation in $CdCl_2$ transformed 16HBE cells.

Bioinformatics analysis of *eIF3* in $CdCl_2$ transformed 16HBE cells

The mRNA, microRNA and lncRNA differentially expression profiling (Table S1,S2, S3) had been done in $CdCl_2$ transformed 16HBE cells in our previous studies. On the basis of these expression profiles, cytoscope software was employed to construct the network diagram of *eIF3*-mRNA co-expression(Table S4). Results showed, in $CdCl_2$ transformed 16HBE cells, *eIF3* was related to the genes involved in the proliferation, differentiation, apoptosis, invasion and migration (Figure 3A). According to the correlation of targets of miRNA-mRNA and miRNA-lncRNA, cytoscope software was used to delineate the ceRNA regulatory network of lncRNA-miRNA-*eIF3*(Table S5). Results showed *hsa-miR-3941* was able to regulate the *eIF3* expression, and *hsa-miR-3941* served as a response element or endogenous RNA (ceRNA). There were 14 lncRNAs in *AL096700.2*, *RP11-213G2.4* and *DHX9P1*, which were involved in the regulation of *eIF3* (Figure 3B).

Correlation between *eIF3* mRNA expression and Cd exposure in rats with chronic Cd exposure

eIF3 mRNA expression increased significantly in rats chronically exposed to low-dose, mid-dose and high-dose Cd, and this increase was Cd-dose

dependent (P<0.05). The *eIF3* mRNA expression in the kidney, heart and lung in low-dose group was comparable to that in control group (P>0.05)(Figure 4). The *eIF3* mRNA expression in the blood was positively related to blood Cd content (r=0.383, p=0.440), Cd content of the urine (r=0.427, P =0.037), liver (r=0.343, P =0.320), kidney (r=0.515, P =0.020), heart (r=0.400, P =0.023) and lung (r=0.402, P=0.280).

Correlation of *eIF3* mRNA expression with with liver and kidney injury in rats chronically exposed to Cd

To explore the correlation of *eIF3* mRNA expression with liver and kidney injury in Cd toxicity, Pearson correlation analysis was used. Results showed the *eIF3* mRNA expression in the rat blood was positively associated with *Scr* (r=0.419, *P* =0.033), *BUN* (r=0.473, *P*=0.017), 24-h Pro (r=0.422, *P*=0.004) and 24-h *Ucr* (*r*=0.475, P =0.016).

Health status of the workers exposed to Cd

The subjects (median age, 31 years) were directly or indirectly exposed to Cd for less than 2 years with no history of exposures to other toxics. Only non-smokers were included in the present study. Urine Cd concentration normalized to the urine creatinine (Cr) showed a normal distribution. The median, maximum and minimum urine Cd concentrations were 1.61, 113.86 and 0.31 μg/g.Cr, respectively. The 25 percentile and 75 percentile of urine Cd concentrations were 0.69 and 9.54 μg/g.Cr, respectively. According to urine Cd concentration, subjects were divided into three groups: ① ≤2 μg/g.Cr, 2-5

μg/g.Cr, and ＞5 μg/g.Cr. The age, gender and years of employment were comparable among three groups, suggesting that our results were not confounded by these factors.

***eIF3* expression was correlated with Cd exposure in Cd-exposed workers**

In order to evaluate whether *eIF3* serves as a biomarker of Cd exposure, the expression of *eIF3* in the blood of Cd-exposed workers was detected by quantitative real-time PCR. According to the urine Cd concentration and blood Cd concentration, these works were divided into 3 groups. The blood *eIF3* expression increased with the increase in urine Cd concentration and blood Cd concentration. The *eIF3* expression in workers with urine Cd concentration at 2-5 μg/g.Cr and >5μg/g.Cr was 1.310 and 5.581 times that in control group (urine Cd concentration: ≤ 2 μg/g.Cr) (P<0.05). A similar finding was identified in blood *eIF3* expression in workers with different blood Cd concentrations (1.659-fold and 6.119-fold) when compared with control group (P<0.05) (Table 2). There was a significant positive correlation of *eIF3* expression with blood Cd concentration (r=0.713, P<0.0001), urine Cd concentration (r=0.459, P=0.003) and urine β_2-*MG* concentration (r=0.501, P=0.001)(Figure 5). These findings indicate that *eIF3* expression is correlated with Cd exposure in Cd-exposed workers.

***eIF3* expression was correlated with the target genes in Cd-exposed workers**

There was a significant positive correlation between *eIF3* expression and the expression of *EGFR, STAT3, FOXC2, VIMENTIN, CASPASE-3* and *BAX*, while a significant negative correlation was noted between *eIF3* expression and expression of *E-CADHERIN, AKT* and *BCL-2* in Cd-exposed workers. In addition, the associations between *eIF3* expression and expression of target genes were further evaluated after adjusting urine Cd concentration and blood Cd concentration (two factors affecting *eIF3* expression and its target gene expression) in Cd-exposed workers. There also was a significant correlation between *eIF3* expression and its target gene expression (Table 3). These findings indicate that *eIF3* expression correlate very well with its target gene expression in Cd-exposed workers.

Discussion

Many cellular and molecular events are involved in the toxic effects of chemical carcinogens in humans[29,30]. It is well recognized that abnormal expression of eukaryotic translation factors facilitates the malignant transformation of primary cells and promotes carcinogenesis[31,32]. Accumulating evidence has demonstrated the important roles of over-expression of translation factors in many cancers including pancreas cancer, colon cancer, breast cancer, lung cancer and prostate cancer[33-35]. Enhanced expression of *eIF3* has been found in a variety of transformed cells, tumor cells and cancer tissues. Our previous study has identified *eIF3* as a novel mouse cadmium-responsive proto-oncogene[25,26]. In addition, our previous results also revealed that the expression of human *eIF3* increased in $CdCl_2$ transformed 16HBE cells at different stages. This study investigated whether *eIF3* modulated cell apoptosis, migration and invasion in Cd-induced toxicity, and examined the *eIF3* expression in Cd exposed rats and workers exposed to Cd. The present study further demonstrated that *eIF3* could be a potential biomarker regulating cell apoptosis, migration and invasion in Cd toxicity and Cd-induced carcinogenesis.

A protein family involved in the translation initiation of proteins, and their changes in the structure and function may increase the sensitivity of cells to transformation, resulting in cell transformation and acquisition of tumorogenic ability. Studies have confirmed that high *eIF3* expression may cause malignant

transformation of cells, and antisense *eIF3* expression reverse the carcinogenesis of Cd-transformed cells[36,37]. However, the consequence of *eIF3* siRNA is still unclear in Cd toxicity. To verify the role of *eIF3* in Cd toxicity, the expression of *eIF3* in untreated 16HBE cells and Cd-induced 35th cells was knocked down with small interfering RNA. Results showed *eIF3* knockdown significantly inhibited the cell apoptosis, migration and invasion in Cd-transformed 16HBE cells. Moreover, *eIF3* knockdown also altered the mRNA expression of genes related to cell apoptosis, migration and invasion (*EGFR, STAT3, FOXC2, VIMENTIN, E-CADHERIN, AKT, BCL-2, CASPASE-3* and *BAX*) in Cd-transformed cells. Our results suggest that *eIF3* silencing may inhibit the apoptosis and compromise the malignancy of Cd-transformed cells.

There were evidences showed that the abnormal genes expression in Cd toxicity were related to the alteration of genetics and epigenetics. O'Connor et al[38] found that *eIF3* mutation altered the initiation codon and the recognition of initiation tRNAs, resulting in abnormal regulation, deficiency and mutation of *eIF3* gene could cause malignant transformation of cells. Nina et al [39] found the mutation of *eIF3-P40* was related to breast cancer and prostate cancer. However, the abnormally expressed mechanisms of *eIF3* are still unclear and have never been reported. In the present study, the exons and promoter of *eIF3* gene were sequenced in untreated cells and Cd-transformed cells, and 1 known SNP was identified in *eIF3* gene, but point mutation was not found. The sequencing and expansion of the predicted CpG of *eIF3* gene failed to show the

DNA methylation. In our previous study, lncRNA, miRNA and mRNA expression profiling was performed and differentially expressed lncRNA, miRNA and mRNA were screened. In this study, bioinformatics analysis was employed to construct the network diagram of *eIF3*-mRNA co-expression and the ceRNA regulatory network of lncRNA-miRNA- *eIF3*. Results showed *hsa-miR-3941* had regulatory effect on *eIF3* in Cd-transformed cells, *hsa-miR-3941* served as a response element or endogenous RNA (ceRNA), and 14 lncRNAs in *AL096700.2, RP11-213G2.4* and *DHX9P1* were related to the regulation of *eIF3*. Above findings suggested that the abnormal expression of *eIF3* in Cd toxicity was related to lncRNA, which was warranted to be confirmed in future studies.

Many cellular and molecular events are involved in the toxic effects of chemical carcinogens[29,40], but few study has been conducted to investigate *eIF3* as a new biomarker of Cd exposure. The present study was undertaken to investigate the role of *eIF3* in Cd toxicity in animals and Cd-exposed workers. The animal model of chronic Cd exposure used in this study was established by continuous intra-peritoneal injection of $CdCl_2$ for 14 weeks. The Cd toxicity was evaluated by the weight coefficient, histo-pathological examination and liver and renal function (*ALT, AST, SCR, BUN* and 24-h Pro) detection. The blood Cd concentration reflects the recent exposure, and urine Cd concentration represents the whole body burden after a long-term exposure, while tissue Cd concentration reflects the Cd accumulation and organ damage[41-43]. In the

present study, the *eIF3* expression in the kidney, liver, lung and heart of Cd-treated rats was positively correlated with the Cd exposure and the severity of organ damage, suggesting that *eIF3* reflects the accumulation of Cd in the body and the severity of organ damage. *eIF3* expression in the body is useful in predicting the Cd-induced toxicity.

In addition, the *eIF3* expression was also detected in the blood and urine of workers chronically exposed to Cd. Results showed a strong positive correlation of blood *eIF3* expression with the urine Cd and the expression of *eIF3* target genes, suggesting that blood *eIF3* expression is potentially a novel biomarker of Cd-exposure in humans. The workers with detectable urine Cd exhibit significantly higher blood *eIF3* expression than those with undetectable urine Cd, suggesting that, even at a lower urine Cd concentration, the blood *eIF3* expression may reflect the alteration in Cd accumulation.

Conclusion

Our study indicates that *eIF3* is able to regulate cellmigration, invasion and apoptosis in the presence of Cd toxicity. In addition, *eIF3* may be a novel and valuable biomarker of Cd toxicity, and may become a significant biomarker for field investigations and risk assessment in humans exposed to occupational and environmental Cd.

Conflict of Interest

The authors have declared that no competing interests exist.

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References

1. W.P. Diao, W.Z. Ni, H.Y. Ma and X.E. Yang, Cadmium pollution in paddy soil as affected by different rice (Oryza sativa L.) cultivars. Bull. Environ. Contam. *Toxicol*., 2005, **75**, 731-738.
2. W.Swaddiwudhipong, P.Mahasakpan, T.Funkhiew and P.Limpatanachote, Changes in cadmium exposure among persons living in cadmium-contaminated areas in northwestern Thailand: A five-year follow-up. *J Med Assoc Thai .,* 2010,**93**, 1217-1222.
3. M.J. Thun, C.G.Elinder and L.Friberg, Scientific basis for an occupational standard for cadmium. *Am J Ind Med.,* 1991,**20**, 629-642 .
4. A.Koyu, A.Gokcimen, F.Ozguner, D.S.Bayram and A.Kocak, Evaluation of the effects of cadmium on rat liver. *Mol. Cell Biochem.* 2006, **20**, 1–5.
5. S.Satarug, S.H. Garrett, M.A. Sens and D.A. Sens, Cadmium, environmental exposure, and health outcomes. *Environ Health Perspect.,* 2010,**118**, 182-190.
6. J.Schöpfer, G.Drasch and G.N. Schrauzer, Selenium and cadmium levels and ratios in prostates, livers, and kidneys of nonsmokers and smokers. *Biol. Trace Elem Res.,* 2010, **134**, 180–187 .
7. R.M. Fay and M.M. Mumtaz, Development of a priority list of chemical mixture occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem Toxicol.,* 1996, **34**, 1163-1165.
8. L.Benbrahim-Tallaa, E.J.Tokar, B.A. Diwan, A.L. Dill, J.F. Coppin and M.P.

Waalkes, Cadmium malignantly transforms normal human breast epithelial cells into a basal-like phenotype. *Environ. Health Perspect.,* **2009**, *117*, 147-152.

9. A.M, Kriegel, Soliman, A.S.; Zhang, Q.; El-Ghawalby, N.; Ezzat, F.; Soultan, A.; Abdel-Wahab, M.; Fathy, O.; Ebidi, G.; Bassiouni, N.; *et al*. Serum cadmium levels in pancreatic cancer patients from the East Nile Delta region of Egypt. *Environ. Health Perspect.* **2006**, *114*, 113–119.
10. K.T. Eriksen , J.Halkjær, J.R. Meliker, J.A. McElroy, M.Sørensen, A. Tjønneland and O.Raaschou-Nielsen. Dietary cadmium intake and risk of prostate cancer: a Danish prospective cohort study. *BMC Cancer.*, 2015, **15**, 177.
11. J.k.Song, H.Luo, X.H.Yin, G.L. Huang, S.Y.Luo, D.R. Lin,Dong B.Yuan, W.Zhang and J.G.Zhu. Association between cadmium exposure and renal cancer risk: a meta-analysis of observational studies. *Scientific RepoRts.,* 5,17976 | DOI: 10.1038/srep17976.
12. H.S.Kim, Y.J.Kim, Y.R.Seo. An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. *J Cancer Prev.,* 2015, **20**, 232-240.
13. J.Huff, R.M. Lunn, M.P.Waalkes, L.Tomatis and P.F. Infante, Cadmium-induced cancers in animals and in humans. *Int. J. Occup. Environ. Health.,* **2007**, *13*, 202–212.
14. The International Agency for Research on Cancer (IARC). *Beryllium,*

Cadmium, Mercury and Exposures in the Glass Manufacturing Industry; World Health Organization International Agency for Research on Cancer: Lyon, France (1993).

15. M.Waisberg, P.Joseph, B.Hale and D. Beyersmann, Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology.,*2003, **192**, 95-117 .

16. M.G. Cherian, P.C. Huang, C.D. Klaassen and Y.P. Liu, D.G. Longfellow and M.P. Waalkes, National Cancer Institute workshop on the possible roles of metallothionein in carcinogenesis.*Cancer Res*., 1993,**53**(4), 922-925

17. S. G. Zimmer, A.DeBenedetti and J. R. Graff, Translational control of malignancy： the mRNA capbinding protein, eIF-4E , as a central regulator of tumor promotion, growth, invasion and metastasis. *Anticancer Res.,* 2000,**20**, 1343-1352.

18. K.Asano, J.Clayton, A.Shalev, and A. G. Hinnebusch, A multifactor complex of eukaryotic initiation factors, eIF1, eIF2, *eIF3*,eIF5, and initiator tRNAMet is an important translation initiation intermediate in vivo. *Genes Dev.,* 2000,**4**, 2534–2546.

19. L. Phan, L.W. Schoenfeld, L. Valasek, A subcomplex of three *eIF3* subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and tRNA(i)Met. *EMBO J*., 2001，**20**,2954-2965.

20. D.Etchison and K.Smith, Variations in cap-binding complexes from

uninfected and poliovirus-infected HeLa cells. *J. Biol. Chem.,* 1990, **265**, 7492-7500.

21. B. J. Lamphear, R.Kirchweger, T.Skern and R.E. Rhoads, Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. *J Biol Chem.,* 1995, **270**, 21975-21983.

22.N.N. Nupponen, K. Porkka and L.Kakkola, Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer. *Am J Pathol*., 1999 ,**154**(6):1777-1783.

23. M.Rothe, Y.Ko, P.Albers and N. Wernert, Eukaryotic initiation factor 3 p110 mRNA is overexpressed in testicular seminomas. *Am J Pathol.,* 2000, **157**, 1597-604 .

24. G. L. Mayeur and J. W. Hershey, Malignant transformation by the eukaryotic translation initiation factor 3 subunit p48 (*eIF3e*). *FEBS Lett.,* 2002, **514**, 49-54.

25. P.Joseph, Y. X. Lei, W. Z. Whong and T.M. Ong, Molecular cloning and functional analysis of a novel cadmium-responsive proto-oncogene. *Cancer Res.,* 2002,**62**, 703-707.

26.Y. X. Lei, P. Joseph and T.M. Ong, Antisense inhibition of translation initiation factor 3 reverses its oncogenic potential. *Teratog Carcinog Mutagen.,* **22**, 2002, 403-409 .

27. Y. X. Lei, L.Wei, M.Wang, G. R.Wu, and M. Li, Malignant transformation

and abnormal expression of eukaryotic initiation factor during human bronchial epithelial cells induced by cadmium chloride. *Biomed. Environ. Sci.*, 2008, 21, 332–338.

28. L. Qian, Y. X. Lei, C. C. He, and Z. N. Lei, Blood Translation Elongation Factor-1δ Is a Novel Marker for Cadmium Exposure. *Int. J. Mol. Sci.,* 2013,**14**, 5182-5197.

29.R.S.Thomas, W.Bao,T.M. M.Chu, T.Bessarabova, Nikolskaya and Y.Nikolsky, et al. Use of short-term transcriptional profiles to assess the long-term cancer-related safety of environmental and industrial chemicals. *Toxicol. Sci.* 2009, **112**, 311–321.

30. B. Vogelstein, and R.W. Kinzler, The mulistep nature of cancer. *Trends Genet.,* 1993,**9**, 138-141 .

31. T. Lee, and J. Pelletier, Eukaryotic initiation factor 4F: A vulnerability of tumor cells. *Future MedChem.,* 2012, **4**, 19-31.

32.N. Sonenberg, Translation factors as effectors of cell growth and tumorigenesis. *Curr OpinmCell Biol.,* 1993*,* **5**, 955-960 .

33. T.Khoury, S. Alrawi, N.Ramnath, Q. Li, M.Grimm and J.Black, Eukaryotic initiation factor-4E and cyclin D1 expression associated with patient survival in lung cancer. *Clin. Lung Cancer*., 2009, **10**, 58–66.

34.C.Siebke, T.C.James, R.Cummins, T.O'Grady, E.Kay and U. Bond, Phage display biopanning identifies the translation initiation and elongation factors (IF1α-3 and eIF-3) as components of Hsp70-peptide complexes in breast

tumour cells. *Cell Stress Chaperones*., 2012, **17**, 145-156.

35.A.Yoshizawa, J.Fukuoka, S.Shimizu, K.Shilo, T.J.Franks and S.M. Hewitt, Overexpression of phospho-eIF4E is associated with survival through AKT pathway in non-small cell lung cancer. *Clin. Cancer Res*., 2010, 6, 240-248.

36.A. Marchetti, F. Buttitta, S. Pellegrini, G. Bertacca and R. Callahan, Reduced expression of INT-6/*eIF3*-p48 in human tumors. *Int J Oncol.*,2001,**18**,175-179

37.J.Eberle, K. Krasagakis and C.E. Orfanos, Translation initiation factor eIF-4A1 mRNA is consistently overexpressed in human melanoma cells in vitro. *Int J Cancer.,* 1997, **71**, 396-401.

38. A. M. Cheng, M. W. Byrom and J. Shelton. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res*., 2005, **33**(4): 1290-1297.

39. M. Kippler, F. Tofail, R. Gardner, A. Rahman, J.D. Hamadani and M. Bottai, Maternal cadmium exposure during pregnancy and size at birth: a prospective cohort study. *Environ Health Perspect*., 2011,**120**(2),284-289.

40.S. Weng, W. Wang, Y. Li and H. Li, Continuous cadmium exposure from weaning to maturity induces downregulation of ovarian follicle development-related SCF/c-kit gene expression and the corresponding changes of DNA methylation/microRNA pattern. *Toxicol Lett.,* 2014, **225**(3),367-77.

41. Y.X. Lei, M.Wang, L.Wei, M.Li and H.Z. Lin, Alterative expression and

sequence analysis of human elongation factor-1δ during malignant transformation of human bronchial epithelial cells induced by cadmium chloride. *Biomed. Environ.Sci*., 2010, **23**, 151-157.

42.M.Ikeda, F. Ohashi, Y. Fukui, S.Sakuragi and J. Moriguchi, Closer correlation of cadmium in urine than that of cadmium in blood with tubular dysfunction markers in urine among general women populations in Japan. *Int. Arch. Occup. Environ. Health*., 2011, **84**, 121–129.

43. L.Järup and A. Akesson, Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol.,* 2009, **238**, 201-208.

Tables

Table 1. Primers used for PCR of selected mRNA and siRNA

mRNAs	Primers
eIF3	F:5’-gCCCTCTCCCCCAACTATgA-3’ R:5’-gTggTTACATCCATggCTTCCT-3’
EGFR	F:5'-GGAGGCAAAGTGCCTATCAA-3' R:5'-AGGTCATCAACTCCCAAACG-3'
STAT3	F:5'-TGTGCGTATGGGAACACCTA-3' R:5'-AGAAGGTCGTCTCCCCCTTA-3'
FOXC2	F:5'-CCTCCTGGTATCTCAACCACA-3' R:5'-GAGGGTCGAGTTCTCAATCCC-3'
VIMENTIN	F:5'-AGTCCACTGAGTACCGGAGAC-3' R:5'-GGTTCCTTTAAGGGCATCCAC-3'
E-CADHERIN	F:5'-AAAGGCCCATTTCCTAAAAACCT-3' R:5' TGCGTTCTCTATCCAGAGGCT-3'
AKT	F:5'-AGCCCACCCTTCAAGCCCCA-3' R:5'-CTGCGCTCGCTGTCCACACA-3'
BCL-2	F:5'-GATAACGGAGGCTGGGATGC-3' R:5'-CAGGCATGTTGACTTCACTTGTG-3'
CAPASE-3	F:5'-AGAGGGGATCGTTGTAGAAGTC-3' R:5'-ACAGTCCAGTTCTGTACCACG-3'
BAX	F:5'-TTGCTTCAGGGGATGATTG-3' R:5'-CAAAGTAGAAAAGGGCGACA-3'
β-actin	F:5'-ACAGAGCCTCGCCTTTGCCGAT-3' R:5'-CTTGCACATGCCGGAGCCGTT-3'
eIF3 siRNA-1	5’-UACUUAAUCUGCGUAAUGGAC-3’ 5’-CCAUUACGCAGAUUAAGUAUA-3’
eIF3 siRNA-2	5’-UCAAAGAAGCUCACAAAGCAC-3’ 5’-GCUUUGUGAGCUUCUUUGACC-3’
eIF3 siRNA-3	5’-ACUCAAAUUCGAAGUACUGUG-3’ 5’-CAGUACUUCGAAUUUGAGUUU-3’
eIF3 siRNA-nc	5’-UUCUCCGAACGUGUCACGUTT-3’ 5’-ACGUGACACGUUCGGAGAATT-3’

Table 2. Blood *eIF3* expression at different Cd exposure levels in Cd-exposed workers.

Exposure to Cd at different levels	N	*eIF3*	F	*P* value
Ucd levels				
① ≤2μg/g Cr	150	0.979±1.007	16.847	$P_{总}$<0.0001
②2-5μg/g Cr	17	1.472±1.149		$P_{①②}$=0.101
③>5μg/g Cr	14	3.304±2.338		$P_{①③}$<0.0001
				$P_{②③}$<0.0001
Urine β_2-MG				
≤500μg/g Cr	147	0.921±0.944	17.684	$P_{总}$<0.0001
500-1000μg/g Cr	19	1.843±1.636		$P_{①②}$=0.001
>1000μg/g Cr	14	3.351±1.987		$P_{①③}$<0.0001
				$P_{②③}$<0.0001
BCd level				
≤2μg/l	131	0.771±0.578	19.134	$P_{总}$<0.0001
2-5μg/l	35	1.421±1.099		$P_{①②}$<0.0001
>5μg/l	15	3.944±2.156		$P_{①③}$<0.0001
				$P_{②③}$<0.0001

Table 3. Correlation Analysis between *eIF3* expression and target gene expression in Cd-exposed workers

Target genes	Correlation coefficient (r)	*P*	Target genes	Correlation coefficient (r)	*P*
EGFR	0.714	<0.0001	*AKT*	-0.417	0.003
STAT3	0.671	0.002	*BCL-2*	-0.687	<0.0001
FOXC2	0.418	<0.0001	*CAPASE-3*	0.684	<0.0001
VIMENTIN	0.758	<0.0001	*BAX*	0.710	0.001
E-CADHERIN	-0.513	0.012			

Figure legends

Figure 1. *eIF3* affects cells migration and invasion in Cd-transformed cells based on transwell and wound-healing assay.

Untreated control 16HBE cells, Cd-transformed 15th passage and Cd-transformed 35th passage cells were knock-down *eIF3*. (A)Representative photographs of migratory cells on the membrane (magnification, 100x). (B) Representative photographs of invasion cells on the membrane (magnification, 100x). The right panels of each row in A and B were the average cell number of triplicate. * P<0.05 vs control cells (t test). (C)The mRNA expression of cell migration and invasion related genes were detected qPCR. * P<0.05 vs control cells (*t* test).

Figure 2. *eIF3* affects cells apoptosis in Cd-transformed cells

Untreated control 16HBE cells, Cd-transformed 15th passage and Cd-transformed 35th passage cells was knock-down *eIF3*. Cell apoptosis was assayed after 72 h treated with *eIF3-siRNA* by flow cytometry using Annexin-V staining(A), data are expressed as cell apoptosis rate(B), and the mRNA expression of cell apoptosis related genes were detected qPCR. * *P*<0.05 vs control cells (*t* tesr).

Figure 3. Bioinformatics analysis of *eIF3* in Cd-induced 35th cells as compared to untreated 16HBE cells.

eIF3-mRNA network (A) and LncRNA-miRNA- *eIF3* network(B) were constructed based on the correlation analysis between differentially expressed lncRNAs, microRNA mRNAs and in Cd-induced 35th 16HBE cells as compared to untreated 16HBE cells.

Figure 4. *eIF3* mRNA expression in kidney, liver, lung and heart of cadmium-exposed

rats using real time qPCR assay.

The gene expression was validated in kidney, liver, lung and heart of cadmium-exposed rats of the control, low dose, Mid-dose and high dose cadmium-exposed rats by qPCR and normalized to that of *β-actin*. Data are expressed as mean ± SE. * $p<0.05$ compared to corresponding control group.

Figure 5. Correlation analysis between *eIF3* expression and Cd concentration in Cd-exposed workers.

Correlation analysis between *eIF3* expression and blood Cd concentration (A), urine Cd concentration (B) and Urine *β2-MG* concentration (C). Blood *eIF3* expression was calculated by the ratio of its expression to that of β-actin. The urine cadmium concentration was normalized by urine creatinine (μg/L.Cr) and urine *β2-MG* (μg/g.Cr). The linear relationship was analyzed by Pearson correlation analysis.

Supplementary tables

Table S1. Differentially Expressed mRNAs

Table S2. Differentially Expressed miRNAs

Table S3. Differentially Expressed LncRNAs

Table S4. eIF3-mRNA co-expression

Table S5. ceRNA (lncRNA-miRNA-eIF3)

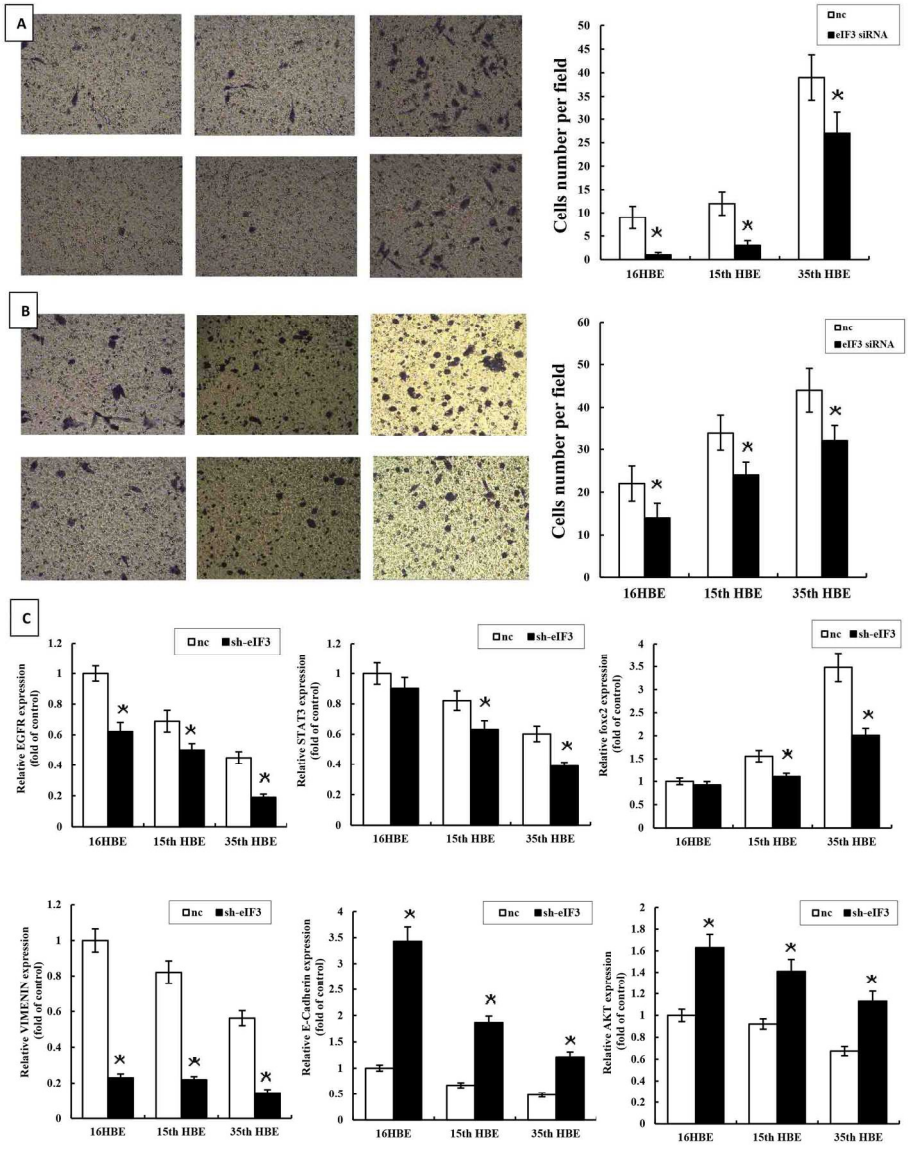


Figure 1. eIF3 affects cells migration and invasion in Cd-transformed cells based on transwell and wound-healing assay.
Untreated control 16HBE cells, Cd-transformed 15th passage and Cd-transformed 35th passage cells were knock-down eIF3. (A)Representative photographs of migratory cells on the membrane (magnification, 100x). (B) Representative photographs of invasion cells on the membrane (magnification, 100x). The right panels of each row in A and B were the average cell number of triplicate. * P<0.05 vs control cells (t test). (C)The mRNA expression of cell migration and invasion related genes were detected qPCR. * P<0.05 vs control cells (t test).
733x962mm (72 x 72 DPI)

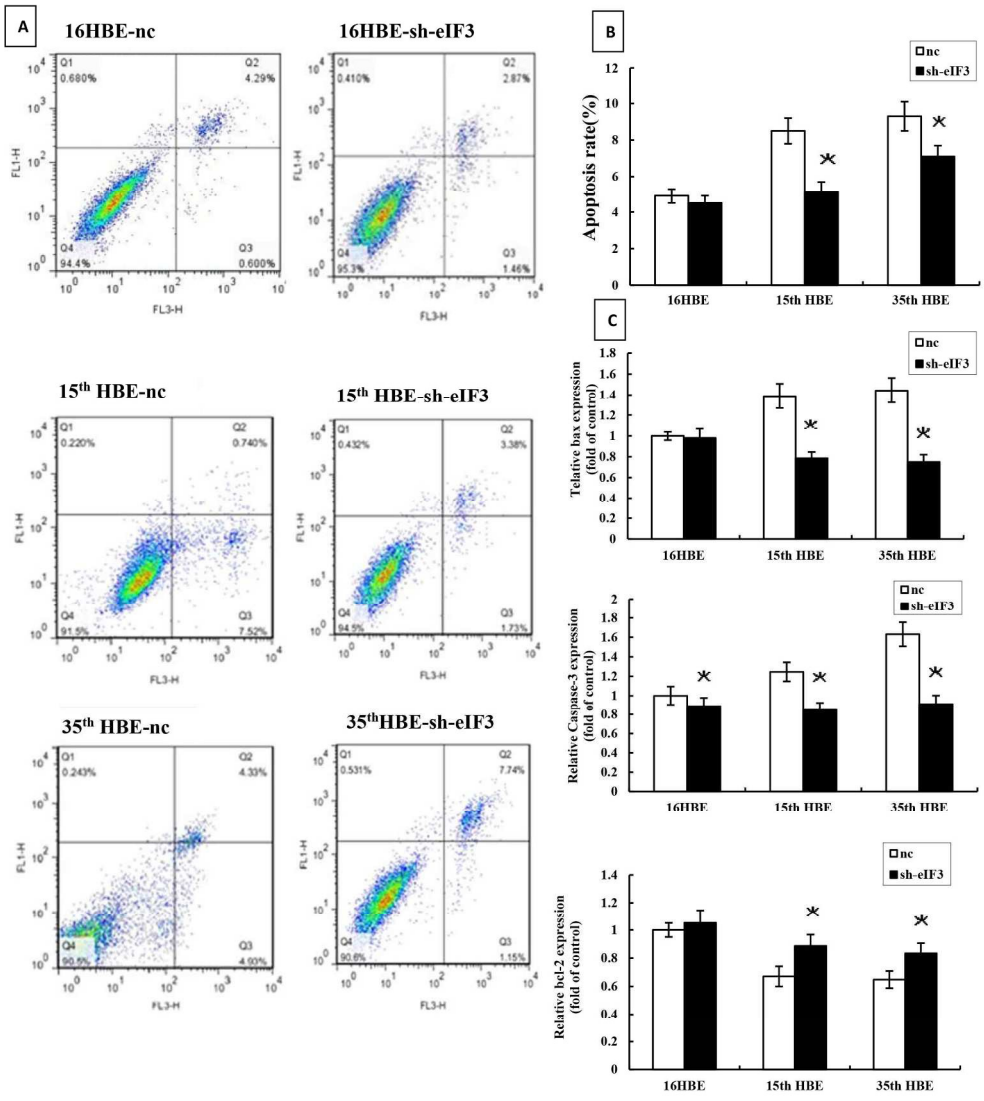
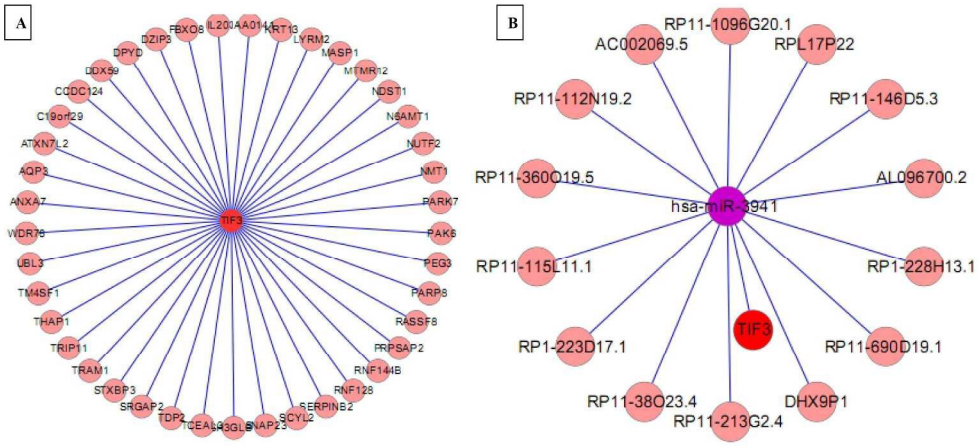


Figure 2. eIF3 affects cells apoptosis in Cd-transformed cells
Untreated control 16HBE cells, Cd-transformed 15th passage and Cd-transformed 35th passage cells was knock-down eIF3. Cell apoptosis was assayed after 72 h treated with eIF3-siRNA by flow cytometry using Annexin-V staining(A), data are expressed as cell apoptosis rate(B), and the mRNA expression of cell apoptosis related genes were detected qPCR. * P<0.05 vs control cells (t tesr).
865x982mm (72 x 72 DPI)



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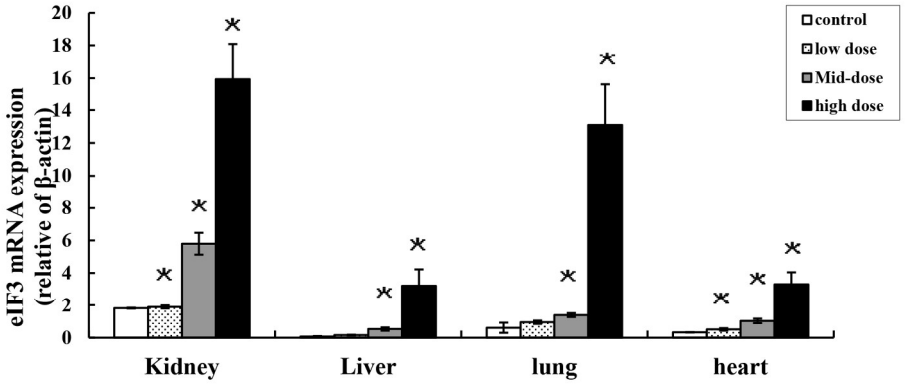

Figure 4. eIF3 mRNA expression in kidney, liver, lung and heart of cadmium-exposed rats using real time qPCR assay.
The gene expression was validated in kidney, liver, lung and heart of cadmium-exposed rats of the control, low dose, Mid-dose and high dose cadmium-exposed rats by qPCR and normalized to that of β-actin. Data are expressed as mean ± SE. * $p<0.05$ compared to corresponding control group.
727x308mm (72 x 72 DPI)

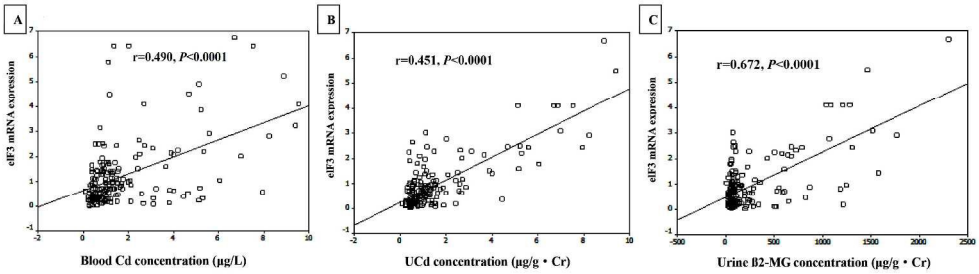

Figure 5. Correlation analysis between eIF3 expression and Cd concentration in Cd-exposed workers. Correlation analysis between eIF3 expression and blood Cd concentration (A), urine Cd concentration (B) and Urine β2-MG concentration (C). Blood eIF3 expression was calculated by the ratio of its expression to that of β-actin. The urine cadmium concentration was normalized by urine creatinine (μg/L.Cr) and urine β2-MG (μg/g.Cr). The linear relationship was analyzed by Pearson correlation analysis.
292x85mm (300 x 300 DPI)