Biomarkers, 2012; 17(1): 78–84

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.639463

informa
healthcare

RESEARCH ARTICLE

Alterations in the expression of translation factors as molecular markers in cadmium-exposed workers

Lian Wei[1], Yi-xiong Lei[1], Lin Wu[2], Min Wang[1], Qian Lu[1], and Chao-cai He[1]

[1]School of Public Health, Guangzhou Medical University, Guangzhou, PR China and [2]Guangzhou Occupational Diseases Prevention and Treatment Hospital, Guangzhou, PR China

Abstract

Eukaryotic translation initiation factor 3 (eIF3) and elongation factor 1δ (eEF-1δ) are novel cadmium (Cd) responsive proto-oncogenes. This research investigated the expression of these genes in Cd-exposed workers (n = 58), and to evaluate their usefulness as biomarkers of Cd exposure. According to urinary Cd concentration, the subjects were divided into four groups (urinary Cd concentration ≥0.1 μg/g.Cr, ≥1.0 μg/g.Cr, ≥5.0 μg/g.Cr and ≥50.0 μg/g.Cr). Subjects exhibited increased severe health problems with higher urinary Cd concentrations. The eIF3 and eEF-1δ expression in the blood were investigated with real-time PCR. PCR data showed a strong positive correlation between blood eEF-1δ and urinary Cd concentrations ($r = 0.788$, $p < 0.01$), and a weak positive correlation between blood eIF3 expression and urinary Cd concentrations ($r = 0.569$, $p < 0.05$). These findings, for the first time, demonstrate that the blood eEF-1δ overexpression can be used as a molecular biomarker of Cd-exposed population.

Keywords: Elongation factor 1δ (eEF-1δ), eukaryotic translation initiation factor 3 (eIF3), cadmium exposure, occupation population, biomarker

Introduction

Cadmium (Cd) and its compounds are heavy metals that find widespread use in industrial applications (Thun et al. 1991; Diao et al. 2005; Swaddiwudhipong et al. 2010). Cd is also widely abundant in air, water, soil and food. Human exposure to Cd through occupational and environmental contaminants is unavoidable. High levels of Cd can be accumulated in various organs, and toxicological responses to Cd exposure include lung, kidney and liver damages (Nordberg et al. 2002; Koyu et al. 2006; Schöpfer et al. 2010). In addition, epidemiologic studies have shown that Cd is associated with many human tumors, including lung, breast, kidney and pancreatic cancers, especially in high-risk population of workers, who are engaged in refining, welding and annealing of Cd (Waalkes 2003; Goyer et al. 2004; Sorahan & Esmen 2004; Kriegel et al. 2006; Benbrahim-Tallaa et al. 2009). Several lines of experimental evidence have shown that Cd is carcinogenic to human and animals (Huff et al. 2007). Based on such studies, the International Agency for Research on Cancer has classified Cd and its compounds, one of the most poisonous metals to human, as carcinogens (Fay & Mumtaz 1996; Huff et al. 2007; IARC 1993). However, the molecular mechanisms underlying the Cd-induced tumorigenesis have not yet been entirely understood.

Translation initiation factors and elongation factors are known to play important regulatory roles in cell growth and tumorigenesis. Mouse eukaryotic translation initiation factor 3 (eIF3 p36) and translation elongation factor 1δ (eEF-1δ p31) have been identified as two novel Cd-responsive proto-oncogenes (Joseph et al. 2002a,b; Lei et al. 2002ab). Our previous studies have found an elevated expression of eIF3 p36 and eEF-1δ p31 at different stages of 16HBE cells transformed by $CdCl_2$ (Lei et al. 2008; Lei et al. 2010b). Recently, we have reported that blood EF-1δ mRNA expression in rats treated with CdCl2 is correlated with Cd-induced liver and kidney toxicity, suggesting that blood EF-1δ expression can be used as a new biomarker for Cd exposure (Lei et al. 2010a).

Address for Correspondence: Yi-xiong Lei, School of Public Health, Guangzhou Medical University, Guangzhou 510182, PR China. Tel.: +86 20 81340196. Fax: +86 20 581340196. E-mail: gz-leizeng@hotmail.com

(Received 01 September 2011; revised 07 November 2011; accepted 07 November 2011)

However, the expression of eIF3 p36 and eEF-1δ p31 in Cd-exposed human population has not yet been studied. In this study, we aimed to determine the expression of the eIF3 p36 and eEF-1δ p31 in Cd-exposed workers in a Cd refinery factory, and to identify the potential utility of eIF3 p36 and eEF-1δ p31 as novel biomarkers of Cd-induced health damages.

Material and methods

Subjects and sample collection

Blood and urine samples were obtained from employees during their physical checkups in a Cd refinery factory with the assistance of Guangzhou Occupational Diseases Prevention and Treatment Hospital. The occupational employees included production workers, machine maintenance workers, product development personnel, management personnel and other personnel engaged in cleaning, service and security et al. The subjects were asked to complete a detailed questionnaire requesting information about employment history, smoking habits, diet and medical history. In addition, they were required to take a comprehensive physical examination. The physical examination included blood pressure, pulse rate, throat and pharynx, lung function, electrocardiogram, liver and kidney ultrasonic, X-ray of cardiopulmonary, blood cells and component, serum alanine aminotransferase (ALT), urinary Cd and creatinine (Cr) concentrations etc. In this study, we excluded subjects who could not provide reliable information on smoking history, those who had a smoking history and those who had a history of kidney or liver diseases. Thus, this study included 58 non-smoking subjects (53 males and 5 females) with ages ranging from 20 to 42 years. Urinary Cd concentration is a good measure of Cd body burden in exposed workers. According to urinary Cd concentrations, subjects were categorized into four groups: ≥0.1 μg/g.Cr, ≥1.0 μg/g.Cr, ≥5.0 μg/g.Cr and ≥50.0 μg/g.Cr.

Serum ALT was measured with an automatic biochemical analyzer (HITACHI7600-020/7170A; Hitachi, Tokyo, Japan), using alanine aminotransferase reagent kit (BIOSION Big-Technology and Science Inc., Beijing, China). An ALT value >40 U/L was defined as abnormal (Prati et al. 2002). Cd in the urine was measured by atomic absorption spectrometry (ZEENIT700; Analytik Jena, Jena, Germany). Levels of urinary Cd below the detection limit were assigned to the value of detection limit (0.003 μg/l). The urinary Cd concentrations were expressed as urinary Cr concentrations/ urinary Cr concentrations (μg/g.Cr).

RNA isolation and primer design

Total RNA was isolated from blood samples with QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA with MMLV reverse transcriptase and oligo-dT primers (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. The primers and probes for real-time PCR were designed with Primer Express 3.0 software and synthesized by Da'an Co. China according to sequences of eIF3 p36 and eEF-1δ p31 from Genbank, and human β-actin was used as the internal standard (Table 1).

Real-time PCR assay

The quantitative detections of eIF3 and eEF-1δ cDNA were performed with Real-time PCR master mix Kit (Da'an Co., Guangzhou, China) in PE 7000 Sequence Detection System (ABI PRISM, Foster City, CA, USA) with human β-actin as internal standard (Livak & Schmittgen 2001). Each 25-μl reaction mixture consisted of 0.5 μl primers (0.5 μmol/L), 1.0 μl Taqman probe (10 pmol/L), 12.5 μl premix EX Taq (2X), then added 2 μl samples (approximately 50 ng cDNA) according to Kit's instructions. After pre-denaturation at 93°C for 2 min, 40 cycles of amplification (denaturation at 93°C for 45s and annealing at 55°C for 45s) were performed for eIF3 and eEF-1δ detection. The standard preparation, negative control, and target samples were all tested in triplicates, and the amounts of target cDNA were automatically calculated according to standard gradient template curve. The PCR products were run on 2% agarose gel to confirm the single target bands, and then the eIF3 and eEF-1δ copies were revised with human β-actin as internal standard in blood samples.

Statistical analysis

Data are presented as mean ± SD ($\bar{x} \pm s$), or median and percentile. The data is changed into normal distribution with logarithm if the original data is positive skewness distribution. The differences among groups were statistically analyzed by analysis of variance followed by Student-Newman-Keuls. Categorical data were compared with χ^2. The relationship between two groups was tested by Pearson or Spearman's correlation, using SPSS 17.0 software. Significance is set at $p < 0.05$.

Results

Health status of subjects exposed to Cd

The subjects (median age, 30 years) were directly or indirectly exposed to Cd for less than 2 years with no history of exposures to other poisons. Only non-smokers

Table 1. Primers and probe sequences for real-time assay.

Genes	Primers and probe sequences	Size
eIF3 p36	F:5′-gccctctcccccaactatga-3′	65 bp
	R:5′-gtggttacatccatggcttcct-3′	
eEF-1δ p31	F:5′-gctacaaacttcctagcacatgagaag-3′	86 bp
	R:5′-ttcatctgctcgtagaatctccttt-3′	
Human β-actin	F:5′-cattgccgacaggatgca-3′	160 bp
	R:5′-ctcctgcttgctgatccacat-3′	
eIF-Probe	5′-FAM-CATgTggTCCTgggCggTggT-TAMARA-3′	
eEF-Probe	5′ -FAM-TggTTCgACAAgTTCAAATATgACg-TAMARA-3′	

eEF, eukaryotic translation elongation factor; eIF, eukaryotic translation initiation factor.

were included in the study. Urinary Cd concentrations normalized to the amount of creatinine (Cr) in the urine showed a positive skewness distribution. The median, maximum and minimum urinary Cd concentrations were 1.61, 113.86 and 0.31 μg/g.Cr, respectively. The 25 percentile and 75 percentile of urinary Cd concentrations were 0.69 and 9.54 μg/g.Cr, respectively. According to urinary Cd concentrations, subjects are categorized into four groups: ≥0.1 μg/g.Cr, ≥1.0 μg/g.Cr, ≥5.0 μg/g.Cr and ≥50.0 μg/g.Cr. The characteristics of the subjects in each group are shown in Table 2. The age, gender and duration of employment did not significantly differ among the four groups, thus suggesting that our study was not confounded by these factors.

The subjects exhibited increased health problems with higher urinary Cd concentrations, which indicated higher Cd accumulation in the body. Inhalation of Cd resulted in pharyngitis, which was diagnosed by subject's complaints of sore throat, and redness and congestion in the back of pharynx seen on physical examination. With a urinary Cd concentration ≥0.1 and <1.0 μg/g.Cr, only 2 (11.8%) of 17 subjects had pharyngitis; with a urinary Cd concentration ≥1.0 and <5.0 μg/g.Cr, 5 (33.3%) of 15 subjects had pharyngitis, and 1 (6.7%) of 15 subjects showed positive red blood cells in urine; with a urinary Cd concentration ≥5.0 and <50.0 μg/g.Cr, 7 (50.0%) of 14 subjects had pharyngitis or congestion, and 6 (42.9%) of 14 subjects showed positive red blood cells in the urine; with a urinary Cd concentration ≥50.0 μg/g.Cr, 8 (66.7%) of 12 subjects had pharyngitis or congestion, and 6 (50.0%) of 12 subjects showed positive red blood cells in the urine; and also with a urinary Cd concentration 5.0 μg/g.Cr and more, 15 (57.7%) of 26 subjects displayed higher ALT activities in the serum comparing with the relative controls (with a urinary Cd concentration <1.0 μg/g.Cr, $p<0.01$; Table 3), indicating that Cd-induced damages in liver, kidney, and respiratory tract occurred in these subjects.

Table 2. Characteristics of the subjects.

Groups with different urinary Cd concentrations (μg/g.Cr)	Cases (male, female)	Age (years)	Duration of employment (years)
0.1~	17 (M16, F1)	30.8 ± 1.43	1.2 ± 0.39
1.0~	15 (M12, F3)	29.5 ± 1.88	1.1 ± 0.35
5.0~	14 (M13, F1)	30.1 ± 2.16	1.2 ± 0.36
50.0~	12 (M11, F1)	30.2 ± 2.04	1.2 ± 0.39

Expression and confirmation of target gene

The representative amplification plot and standard curve of the real-time PCR was shown in Figure 1. The standard curve showed a linear relationship between the Ct (cycle threshold) value and initial amount of DNA, and had a correlation coefficient of 0.999. Figure 2 showed the representative amplification plot and identification of target eIF3 and eEF-1δ genes with real-time PCR assay in the workers exposed to Cd. The agarose gels showed single target bands of PCR products, confirming that the target genes were amplified by the real-time PCR.

Analysis of blood target gene expressions

According to urinary Cd concentrations, the subjects were divided into four groups as shown in Table 4. The eIF3 and eEF-1δ expressions in the blood increased with the increased urinary Cd concentrations (Table 4). The eEF-1δ level was significantly higher in groups with urinary Cd concentrations ≥1.0 μg/g.Cr, ≥5.0 μg/g.Cr and ≥50.0 μg/g.Cr, with a 2-fold, 7-fold, and 34-fold increase, respectively, compared with the control group (urinary Cd concentration <1.0 μg/g.Cr) ($p<0.01$). A similar finding but to a less extent was identified in blood eIF3 expression. A significant but weak positive correlation between blood eIF3 expression and urinary Cd concentrations was identified in correlation analysis ($r=0.569$, $p<0.05$) (Figure 3A). A strong positive correlation between blood eEF-1δ expression and urinary Cd concentrations was found ($r=0.788$, $p<0.01$) (Figure 3B).

Discussion

This study investigates the expression of eIF3 and eEF-1δ in a population of workers exposed to Cd. We have examined the blood eIF3 and eEF-1δ expression and urinary Cd concentrations in these subjects, and have found a strong positive correlation between blood eEF-1δ and urinary Cd concentrations, and a weak positive correlation between blood eIF3 expression and urinary Cd concentrations, suggesting that blood eEF-1δ is potentially a good biomarker of Cd-exposed population.

Tobacco smoking is a major source of Cd exposure in the general population. To exclude the confounding effects of smoking, current and former smokers as well as those who cannot provide a reliable smoking history are excluded in our study. Therefore, our results are unlikely confounded by smoking status of the subjects,

Table 3. Cd-induced health problems in different Cd-exposed groups.

Groups with different urinary Cd concentrations (μg/g.Cr)	Cases	Health problems		
		Pharyngitis (%)	Positive RBC in urine (%)	Higher ALT activity (%)
0.1~	17	2 (11.8)	0 (0)	1 (5.9)
1.0~	15	5 (33.3)*	1 (6.7)	2 (13.3)
5.0~	14	7 (50.0)*	6 (42.9)*	6 (42.9)*
50.0~	12	8 (66.7)**	6 (50)**	9 (75.0)**

ALT, alanine aminotransferase; RBC, red blood cells.
*$p<0.05$ and **$p<0.01$ vs control (urinary Cd concentration < 1.0 μg/g.Cr) by χ^2 test.

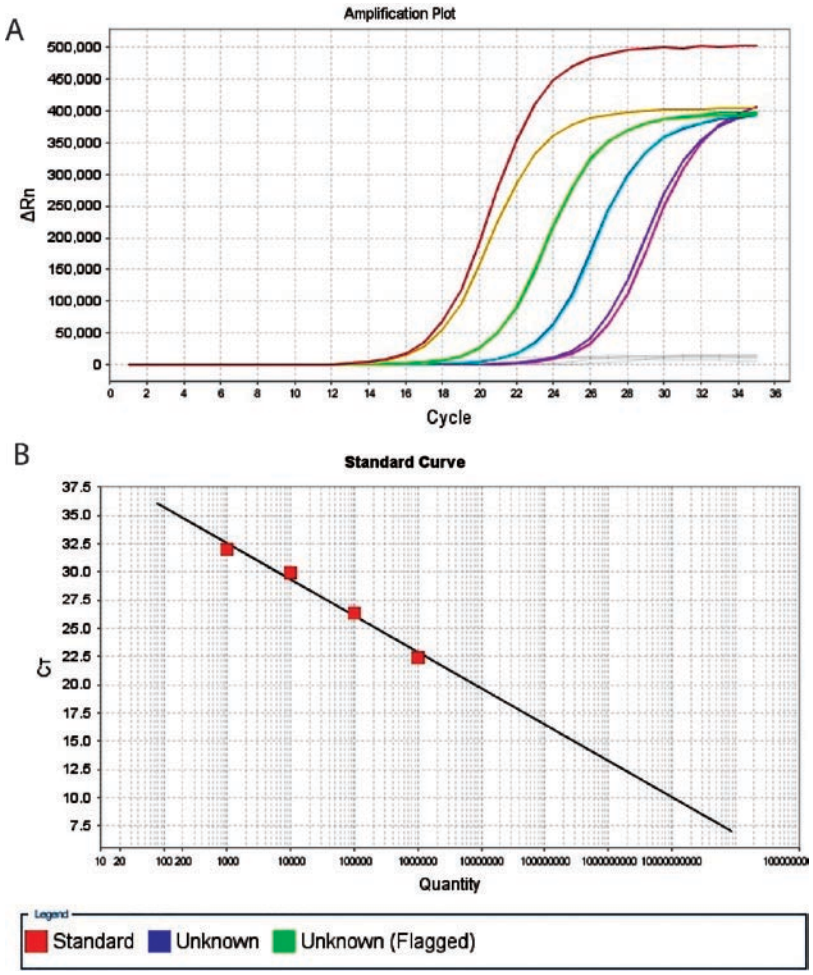

Figure 1. A typical amplification plot (A) and the standard curve (B) of the real-time PCR. (A) The amplification plot showing the change in fluorescence (ΔRn) plotted versus cycle number. (B) the standard curve showing the Ct value plotted versus the initial amount of DNA. The Ct (cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross the threshold in a quantitative PCR reaction.

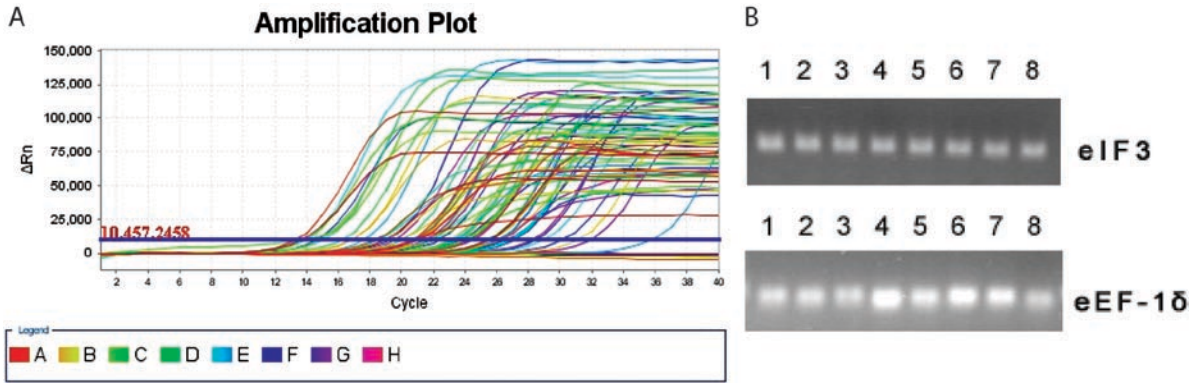

Figure 2. The representative amplification plot (A) and agarose gel analysis (B) of target gene from real-time PCR. (A) The amplification plot of eIF3 and eEF-1δ genes showing the change in fluorescence (ΔRn) plotted versus cycle number. (B) Fluorescent bands in the agarose gels showing positive results for eIF3 and eEF-1δ genes. Lanes 1–8: different single target cDNA bands.

since only non-smokers are included in the study. In addition, it is unlikely that other confounding factors such as sex, age and duration of employment affect our results, since no significant differences in sex, age and duration of employment are identified among the four groups.

A biomarker is to be considered as a good measure of the biological effect of a certain exposure if the changing of the biomarker can reflect the corresponding change in the level of exposure. In this sense, the blood eEF-1δ expression is a good biomarker since its expression level is strongly correlated with the urinary

Table 4. Blood eIF3 and eEF-1δ expressions and urinary Cd concentrations in Cd-exposed subjects.

Groups with different urinary Cd concentrations (μg/g.Cr)	Cases (male, female)	Median urinary Cd concentration (μg/g.Cr)	Median blood eIF3 concentration	Median of blood eEF-1δ concentration
0.1~	17 (M16, F1)	0.61	0.124	0.111
1.0~	15 (M12, F3)	1.76*	0.165	0.210*
5.0~	14 (M13, F1)	10.79**	0.357*	0.781**
50.0~	12 (M11, F1)	75.20**	1.155**	3.775**

Urinary Cd concentration was normalized by creatinine (Cr) concentration. Blood eIF3 and eEF-1δ gene expression levels were calculated by the ratio of their expression to that of β-actin.
eEF, eukaryotic translation elongation factor; eIF, eukaryotic translation initiation factor.
*$p<0.05$ and **$p<0.01$ vs control (urinary Cd concentration < 1.0 μg/g.Cr). Data were changed into normal distribution with logarithm and statistically analyzed by analysis of variance followed by Student-Newman-Keuls.

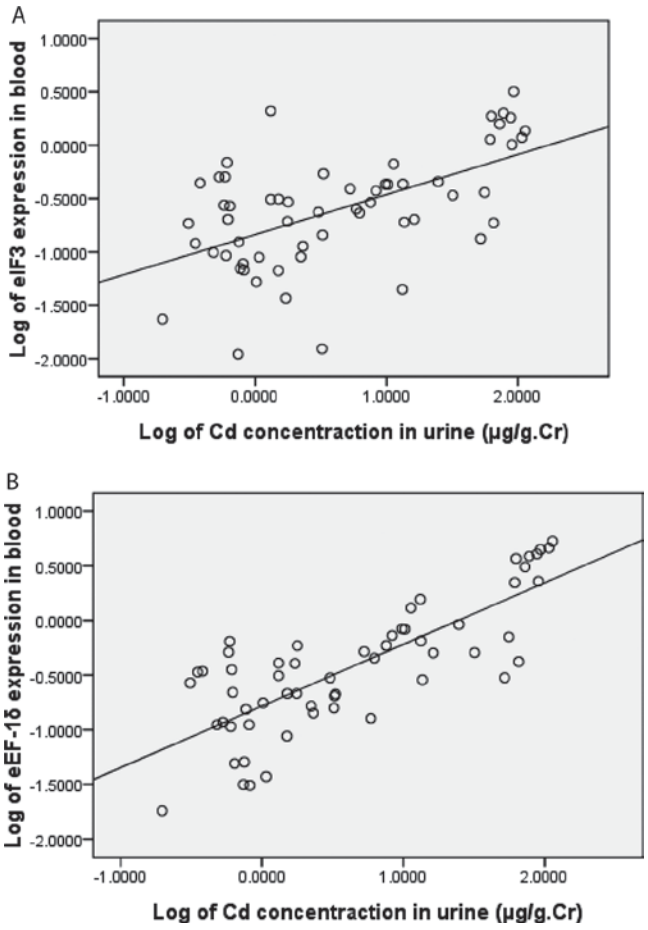

Figure 3. The correlation analysis between blood eIF3 and eEF-1δ expression levels and urinary Cd concentrations in Cd-exposed subjects. Urinary Cd concentration was normalized by creatinine (Cr) concentration, and blood eIF3 and eEF-1δ gene expression levels were calculated by the ratio of their expressions to that of β-actin. The logarithm of blood eIF3 (A) and eEF-1δ (B) gene expression levels was plot against the logarithm of urinary Cd concentration, and the linear relationship was analyzed by Person correlation. For eIF3 gene, $r=0.569$, $p<0.05$; for eEF-1δ gene, $r=0.788$, $p<0.01$.

Cd concentration. Subjects with urinary Cd concentrations (>1.0 μg/g.Cr), exhibit significantly higher blood eEF-1δ expression levels than those with urinary Cd concentrations (>0.1 μg/g.Cr) (Table 4), suggesting that even at a lower range of urinary Cd concentrations, the change in blood eEF-1δ expression levels can reflect the alterations in the Cd accumulation. In contrast, we do not identify a statistically significant difference in the eIF3 expression between the two groups with lower urinary Cd concentrations, suggesting that eIF3 is not as sensitive as eEF-1δ to function as a biomarker of Cd exposure.

We also find that subjects exhibit more severe health problems at higher urinary Cd concentrations. With urinary Cd concentrations more than 5.0 μg/g.Cr, more subjects had symptoms of pharyngitis or congestion, hematuria, and higher alanine transaminase activities, suggesting that Cd-induced damages in kidney, liver, and respiratory tract occur in these subjects. Since there is a strong correlation between blood eEF-1δ expression and urinary Cd concentrations, it is reasonable to conclude that eEF-1δ expression is a good biomarker of Cd-induced health damages. In addition, we also find that blood eEF-1δ expression in rats treated with Cd is a potential new biomarker for chronic Cd exposure (Lei et al. 2010a).

Recently, it has been reported that the mouse eIF3 and eEF-1δ are identified as two Cd-responsive proto-oncogenes (Joseph et al. 2002a,b; Lei et al. 2002a,b; Joseph et al. 2004). In addition, we have previously found that the expression of human eIF3 and eEF-1δ is elevated at different stages of 16HBE cells transformed by $CdCl_2$ (Lei et al. 2008; Lei et al. 2010b). It is known that modifications in the translational machinery of cells due to changes in both eukaryotic translation initiation factors and elongation factors can result in susceptibility to transformation and the acquisition of transformed and oncogenic properties in cells (Sonenberg 1993; Beyersmann & Hechtenberg 1997). Enhanced expression of eukaryotic translation initiation factor 3 (eIF3) subunits has been found in a variety of transformed cells, tumor cells and tumor tissues (Nupponen et al. 1999; Mayeur & Hershey 2002). Similarly, levels of elongation factors such as EF-1α and EF-1γ are elevated in tumor tissues of the pancreas, colon, breast, lungs, prostate and stomach compared to those in normal tissues (Grant et al. 1992; Tatsuka et al. 1992; Ender et al. 1993). Since epidemiologic studies have demonstrated that Cd is associated with several human tumors, and our study shows that blood eEF-1δ expression is strongly correlated with Cd concentrations accumulated in the body, it is likely that eEF-1δ can be used as a biomarker of Cd-induced carcinogenesis.

Urinary Cd concentration is a good indicator of Cd body burden in currently exposed workers, and is widely used as a biological measure for long-term Cd exposure. However, urinary Cd concentration cannot reflect the cellular mechanisms underlying Cd-induced cytotoxicity. In contrast, the cellular expression level of eEF-1δ is known to be a critical determinant in regulating the mRNA translation, and alterations in the cellular translational machinery have been identified to be responsible for Cd-induced cytotoxicity and cell death (Waisberg et al. 2003). In agreement with this concept, we find that blood eEF-1δ expression level is associated with the extent of health damages, suggesting that Cd-induced cytotoxicity may be mediated through an elevation of eEF-1δ expression. In addition, alterations in eEF-1δ expression may be prominent in Cd-exposed population at the early stage before health problems occur. However, in this study, though we select the control group with urinary Cd concentrations similar to those in the normal unexposed population (<1 μg/g.Cr), we have not investigated the eEF-1δ expression in the unexposed population. The study is worthy of further investigation with a large sample size.

In summary, we identify that blood eEF-1δ expression is strongly correlated with urinary Cd concentration. These findings, for the first time, demonstrate that the blood eEF-1δ overexpression can be used as a new and potential molecular biomarker Cd-exposed population.

Declaration of interest

This work was supported by grants from the National Natural Science Foundation of China (81072322, 30771781), the Natural Science Foundation of Guangdong Province, China (06022672), and the Science and Technology Key Project of Guangdong Municipality, China (2003Z2-E0191, 2003Z2-E0192). The authors report no conflict of interest.

References

Benbrahim-Tallaa L, Tokar EJ, Diwan BA, Dill AL, Coppin JF, Waalkes MP. (2009). Cadmium malignantly transforms normal human breast epithelial cells into a basal-like phenotype. *Environ Health Perspect* 117:1847–1852.

Beyersmann D, Hechtenberg S. (1997). Cadmium, gene regulation, and cellular signalling in mammalian cells. *Toxicol Appl Pharmacol* 144:247–261.

Diao WP, Ni WZ, Ma HY, Yang XE. (2005). Cadmium pollution in paddy soil as affected by different rice (Oryza sativa L.) cultivars. *Bull Environ Contam Toxicol* 75:731–738.

Ender B, Lynch P, Kim YH, Inamdar NV, Cleary KR, Frazier ML. (1993). Overexpression of an elongation factor-1 gamma-hybridizing RNA in colorectal adenomas. *Mol Carcinog* 7:18–20.

Fay RM, Mumtaz MM. (1996). Development of a priority list of chemical mixtures occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem Toxicol* 34:1163–1165.

Goyer RA, Liu J, Waalkes MP. (2004). Cadmium and cancer of prostate and testis. *Biometals* 17:555–558.

Grant AG, Flomen RM, Tizard ML, Grant DA. (1992). Differential screening of a human pancreatic adenocarcinoma lambda gt11 expression library has identified increased transcription of elongation factor EF-1 alpha in tumour cells. *Int J Cancer* 50:740–745.

Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF. (2007). Cadmium-induced cancers in animals and in humans. *Int J Occup Environ Health* 13:202–212.

IARC. (1993). Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, Lyon, France. *Intl Agency Res Cancer* 58:119–238.

Joseph P, Lei YX, Ong TM. (2004). Up-regulation of expression of translation factors–a novel molecular mechanism for cadmium carcinogenesis. *Mol Cell Biochem* 255:93–101.

Joseph P, Lei YX, Whong WZ, Ong TM. (2002a). Molecular cloning and functional analysis of a novel cadmium-responsive proto-oncogene. *Cancer Res* 62:703–707.

Joseph P, Lei YX, Whong WZ, Ong TM. (2002b). Oncogenic potential of mouse translation elongation factor-1 delta, a novel cadmium-responsive proto-oncogene. *J Biol Chem* 277:6131–6136.

Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A. (2006). Evaluation of the effects of cadmium on rat liver. *Mol Cell Biochem* 284:81–85.

Kriegel AM, Soliman AS, Zhang Q, El-Ghawalby N, Ezzat F, Soultan A, Abdel-Wahab M, Fathy O, Ebidi G, Bassiouni N, Hamilton SR, Abbruzzese JL, Lacey MR, Blake DA. (2006). Serum cadmium levels in pancreatic cancer patients from the East Nile Delta region of Egypt. *Environ Health Perspect* 114:113–119.

Lei YX, Chen JK, Wu ZL. (2002a). Blocking the translation elongation factor-1 delta with its antisense mRNA results in a significant reversal of its oncogenic potential. *Teratog, Carcinog Mutagen* 22:377–383.

Lei YX, Joseph P, Ong TM. (2002b). Antisense inhibition of translation initiation factor 3 reverses its oncogenic potential. *Teratog, Carcinog Mutagen* 22:403–409.

Lei YX, Lu Q, He CC, Lei ZM. (2010a). Blood TEF-1δ expression as a new biomarker for cadmium exposure with chronic research in vivo. *Proceedings of the 5th International Academic Conference on Environmental and Occupational Medicine*:239–243.

Lei YX, Wei L, Wang M, Wu GR, Li M. (2008). Malignant transformation and abnormal expression of eukaryotic initiation factor in bronchial epithelial cells induced by cadmium chloride. *Biomed Environ Sci* 21:332–338.

Lei YX, Wang M, Wei L, Lu X, Lin HZ. (2010b). Alternative expression and sequence of human elongation factor-1 delta during malignant transformation of human bronchial epithelial cells induced by cadmium chloride. *Biomed Environ Sci* 23:151–157.

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.

Mayeur GL, Hershey JW. (2002). Malignant transformation by the eukaryotic translation initiation factor 3 subunit p48 (eIF3e). *FEBS Lett* 514:49–54.

Nordberg G, Jin T, Bernard A, Fierens S, Buchet JP, Ye T, Kong Q, Wang H. (2002). Low bone density and renal dysfunction following environmental cadmium exposure in China. *Ambio* 31:478–481.

Nupponen NN, Porkka K, Kakkola L, Tanner M, Persson K, Borg A, Isola J, Visakorpi T. (1999). Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer. *Am J Pathol* 154:1777–1783.

Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. (2002). Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 137:1–10.

Schöpfer J, Drasch G, Schrauzer GN. (2010). Selenium and cadmium levels and ratios in prostates, livers, and kidneys of nonsmokers and smokers. *Biol Trace Elem Res* 134:180–187.

Sonenberg N. (1993). Translation factors as effectors of cell growth and tumorigenesis. *Curr Opin Cell Biol* 5:955–960.

Sorahan T, Esmen NA. (2004). Lung cancer mortality in UK nickel-cadmium battery workers, 1947-2000. *Occup Environ Med* 61:108–116.

Swaddiwudhipong W, Mahasakpan P, Funkhiew T, Limpatanachote P. (2010). Changes in cadmium exposure among persons living in cadmium-contaminated areas in northwestern Thailand: a five-year follow-up. *J Med Assoc Thai* 93:1217–1222.

Tatsuka M, Mitsui H, Wada M, Nagata A, Nojima H, Okayama H. (1992). Elongation factor-1 alpha gene determines susceptibility to transformation. *Nature* 359:333–336.

Thun MJ, Elinder CG, Friberg L. (1991). Scientific basis for an occupational standard for cadmium. *Am J Ind Med* 20:629–642.

Waalkes MP. (2003). Cadmium carcinogenesis. *Mutat Res* 533: 107–120.

Waisberg M, Joseph P, Hale B, Beyersmann D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology* 192:95–117.