# Alterative Expression and Sequence of Human Elongation Factor-1δ during Malignant Transformation of Human Bronchial Epithelial Cells Induced by Cadmium Chloride[1]

YI-XIONG LEI[#,*], MIN WANG[&], LIAN WEI[#], XI LU[#], AND HUA-ZHAO LIN[#]

*[#]Department of Preventive Medicine, Guangzhou Medical University, Guangzhou 510182, Guangdong, China; [&]Institute for Chemical Carcinogenesis, Guangzhou Medical University, Guangzhou 510182, Guangdong, China*

**Objective** To study the alternative expression and sequence of human elongation factor-1δ (human EF-1δ p31) during malignant transformation of human bronchial epithelial cells induced by cadmium chloride ($CdCl_2$) and its possible mechanism. **Methods** Total RNA was isolated at different stages of transformed human bronchial epithelial cells (16HBE) induced by $CdCl_2$ at a concentration of 5.0 μM. Special primers and probe for human EF-1δ p31 were designed and expression of human EF-1δ mRNA from different cell lines was detected with fluorescent quantitative PCR technique. EF-1δ cDNA from different cell lines was purified and cloned into pMD 18-T vector followed by confirming and sequencing analysis. **Results** The expressions of human EF-1δ p31 at different stages of 16HBE cells transformed by $CdCl_2$ was elevated ($P<0.01$ or $P<0.05$). Compared with their corresponding non-transformed cells, the overexpression level of EF-1δ p31 was averagely increased 2.9 folds in Cd-pretransformed cells, 4.3 folds in Cd-transformed cells and 7.2 folds in Cd-tumorigenic cells. No change was found in the sequence of overexpressed EF-1δ p31 at different stages of 16HBE cells transformed by $CdCl_2$. **Conclusion** Overexpression of human EF-1δ p31 is positively correlated with malignant transformation of 16HBE cells induced by $CdCl_2$, but is not correlated with DNA mutations.

**Key words**: Human elongation factor-1δ; Cadmium chloride; Human bronchial epithelial cells; Cell transformation; Sequencing analysis

## INTRODUCTION

Metallic cadmium (Cd) and its related compounds are known environmental and occupational metals[1]. A large number of workers are potentially exposed to Cd in a variety of occupational settings[2-3]. Significant human exposure to Cd also occurs through the ingestion of Cd-contaminated food[4-5]. Considerable quantities of cadmium compounds have been detected in tissues and various organs of individuals exposed to the metal. The toxicological responses of exposure to Cd include kidney and liver damage, respiratory diseases, neurological disorders, and bone effects[6-8]. Available experimental and epidemiological data show that Cd and its compounds are carcinogenic to experimental animals and humans. For example, lung and prostate have been reported as targets for cancer development in people exposed to Cd[9-10]. Cadmium can induce kidney, prostate, and testicular cancers in rats and mice[11]. Based on the results of epidemiological and experimental studies, Cd and compounds containing Cd were classified as human carcinogens in 1993 by the International Agency for Research on Cancer (IARC)[12]. However, the molecular mechanism underlying Cd carcinogenesis remains unknown.

Recent developments in oncogenomics have illustrated that carcinogen-induced changes in gene expression help to understand the potential cellular and molecular mechanisms of chemical carcinogenesis. In recent years, Dr. Joseph and Lei *et al* have identified the mouse translation elongation factor-1δ (TEF-1δ p31) as a novel cadmium-responsive proto-oncogene, and the mouse TEF-1δ p31 exhibits the highest similarity to human elongation factor-1δ (human EF-1δ p31), 92% and 76% similarity to the amino acid and the nucleotid sequence, respectively[13-14]. It is worth determining if

[1]Supported by the National Natural Science Foundation of China (No. 30771781), and the Natural Science Foundation of Guangdong Province (No.06022672).

[*]Corresponding should be addressed to: Yi-Xiong LEI, Department of Preventive Medicine, Guangzhou Medical University, Guangzhou 510182, Guangdong, China. Fax: 020-81340196, E-mail: gz-leizeng@163.com

Biographical note of the first author: Yi-Xiong LEI, male, born in 1960, Ph.D., professor, majoring in occupational and environmental health.

0895-3988/2010
CN 11-2816/Q

overexpression and oncogenic potential of human EF-1δ p31 are present during malignant transformation of human cells induced by Cd.

Malignant transformation in morphological cells coupled with soft agar / nude mouse assay is a useful approach to investigate human carcinogenic potential of environmentally and occupationally related agents[15]. Recently, we have established a model system of malignant cell transformation with cadmium chloride ($CdCl_2$) in human bronchial epithelial cells (16HBE) which are the main target cells of Cd and its compounds. It is relatively sensitive, inexpensive, and easy to perform. Moreover, it enables us to study the mechanistic processes during carcinogenesis[16]. In this study, alterative expression and sequence of human EF-1δ p31 were observed and the possible underlying mechanism was assessed at different stages of 16HBE cells induced by $CdCl_2$.

## MATERIALS AND METHODS

### *Preparation of Different 16HBE Cell Lines*

16HBE cells immortalized by SV40 large T antigen were kindly provided by Dr. Xu (Guangzhou Institute of Respiratory Diseases, China)[17]. Morphological transformation of 16HBE cells induced by $CdCl_2$ was performed as previously described[16]. Briefly, 16HBE cells were treated several times at different concentrations of $CdCl_2$. Medium was used as a solvent control and distilled water was used as a negative control. Cytotoxicity and cell transformation assays were performed as previously described[18]. The transformed cells were identified by assays for anchorage-independent growth in soft agar and tumorigenicity in nude mice. Different 16HBE cell lines induced by $CdCl_2$ at a moderate cytotoxicity (5.0 μmol), which gave approximately 50% of the relative colony forming efficiencies (RCFE), were used in the present study.

### *RNA Isolation and Primer (Probe) Sequences*

Media from different 16HBE cell lines including non-transformed, Cd-pretransformed, Cd-transformed, and Cd-tumorigenic cells, were completely aspirated and rinsed two times with 1×PBS. Total RNA was isolated from the cells using a TRIzol kit (Gibco BRL, USA) according to its manufacturer's instructions. Purity and integrity of total RNA, used for reverse transcription of mRNA to cDNA, were analyzed by spectrophotometry (Eppendorf, Germany) and agarose gel electrophoresis, respectively. For the detection of EF-1δ p31 expression by quantitative assays, primers of human EF-1δ p31 (GenBank accession number Z21507) and corresponding probes were designed with Primer Express 2.0 software and synthesized by Da'an Co. China (Table 1).

TABLE 1

Primers and Probe of Human EF-1δ p31 for Fluorescent Quantitative-Polymerase Chain

| | Reaction (FQ-PCR) Assay Sequences of Primers and Probe | Length of Products |
|---|---|---|
| Sense | 5′-gCTACAAACTTCCTAgCACATgAgAAg-3′ | 86 bp |
| Antisense | 5′-TTCATCTgCTCgTAgAATCTCCTTT-3′ | |
| Probe | 5′-FAM-TggTTCgACAAgTTCAAATATgACg-TAMARA-3′ | |

### *Standards for Positive Templates*

Human EF-1δ cDNA generated by reverse transcription from target RNA was amplified as the standards for positive templates in PE9600 PCR machine (ABI PRISM, USA). Five×PCR buffer containing10 mmol Tris-HCl (pH 8.0), 50 mmol KCl, 2 mmol $MgCl_2$ was mixed with 20 μmol Taq DNA polymerase, 10 mmol dNTPs and 25 μmol primers, then 5 μL of human EF-1δ cDNA template (corresponding to 1.25 μg RNA) was added to the master mix. PCR amplification was performed with a pre-denaturation at 93 ℃ for 2 min, followed by 40 cycles of denaturation at 93 ℃ for 1 m, annealing at 55 ℃ for 1 min and extension at 72 ℃ for 1 min. Copies (copies/μL) of EF-1δ cDNA were calculated according to the $OD_{260}$ and fragment length of PCR products, and diluted to the gradient concentrations of $10^7$, $10^6$, $10^5$, $10^4$, $10^3$ copies/μL for the standard gradient templates. Tri-distilled water was used as a negative control.

### *Detection of Human EF-1δ by FQ-PCR Assay*

*Detection of human EF-1δ cDNA* Human EF-1δ cDNA was detected by fluorescent quantitative-polymerase chain reaction (FQ-PCR) assay in a PE 7000 sequence detection system (ABI PRISM, USA) as previously described[19]. Briefly, a commercially available FQ-PCR master mix (Da'an Co. China) containing 5×PCR buffer, 20 μmol Taq DNA polymerase, 10 mmol dNTPs, was mixed with 25 μmol primers, 20 μmol probe and 5 μL of human EF-1δ cDNA template (corresponding to 1.25 μg RNA). Then, 50 μL of total reaction system was

performed with pre-denaturation at 93 ℃ for 2 min, followed by 40 cycles of denaturation at 93 ℃ for 45 s, and annealing at 55 ℃ for 45 s. When the samples were examined by FQ-PCR, EF-1δ cDNA standard gradient templates and blank reagents were simultaneously amplified. Each sample was examined in triplicate. Fluorescence was measured at the end of annealing-extension phase of each cycle. The PE 7000 sequence detection system (ABI PRISM, USA) was used to detect the fluorescent intensity at 518 nm wavelength and the amount of target cDNA (copies/μg RNA) was calculated according to the standard gradient template curve and compared with its threshold value which was set for the fluorescence of all samples. The reaction cycle at which the PCR product exceeded this fluorescence threshold was identified as the threshold cycle (CT).

*Calculation of human EF-1δ cDNA* The amount of target cDNA (copies/μg RNA) was calculated according to standard gradient template curve from the following formula: $C=Q\times V_{cDNA} / (V_{RNA}\times L_{RNA})$, where C represents human EF-1δ cDNA copies, Q represents the amount of target cDNA examined, $V_{cDNA}$ represents the volume of cDNA template, $V_{RNA}$ represents the volume of RNA template for reverse transcript, and $L_{RNA}$ represents the concentration of RNA template.

*Cloning and Sequencing of Human EF-1δ*

Four lines of single-cell cloning were established from non-transformed, Cd-transformed and Cd-tumorigenic cells, separately, as previously described[20]. Total RNA was isolated from the cells with a TRIzol kit and two fragments of human EF-1δ cDNA were obtained by RT-PCR with the specific primers (F1: TTTCATCAgTCTTCCCgCgT, R1: ggCTCACACCTgTAATCC CAA, 166 bp; F2: TTT CATCAgTCTTCCCgCgT, R2: CTTgTTgAAAgCTgCgATATCg, 910 bp). PCR amplification products were purified by electrophoresis on an agarose gel and specific DNA fragments were recollected. The target DNA fragment was cloned into the pMD18-T-vector using the T4-DNA ligase (TaKaRa Biomedicals) following its manufacturer's instructions. The inserted plasmid pMD18-T with EF-1δ gene was transformed into competent cells (*E. Coli* DH5α). Antibiotic selection and blue / white color selection methods were used in the transformant-selecting process. Several clones selected at random were cultured in a LB medium. After the fused plasmids were isolated, sub-cloning of the EF-1δ fragments was confirmed with restriction enzyme (*Bam*HI, *Eco*RI, and *Hin*dIII) digestion and gel electrophoresis analysis of the digested plasmid. Double-stranded sequencing of the EF-1δ was preformed in Da'an Co. China.

*Statistical Analysis*

Student's *t*-test was used to analyze the data from the two groups. Differences among multi-groups were analyzed by one-way ANOVA or Dunnett test using Statistical Package for the Social Sciences. Data were presented as mean±SD. $P<0.05$ was considered statistically significant.

## RESULTS

*Alterative Expression of EF-1δ in 16HBE Cells Transformed by Cd*

*Kinetic curves for positive standard human EF-1δ* Kinetic curves for positive gradient standard human EF-1δ cDNA and negative quality control plot for fluorescent quantitative PCR are shown in Fig.1. The correlation coefficient between Ct value and

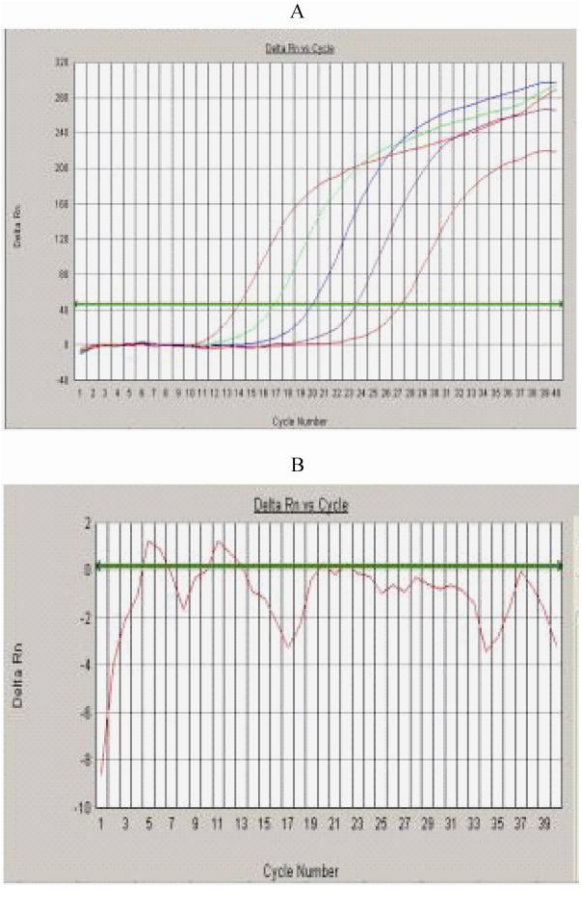


FIG. 1. Kinetic curves for positive gradient standard human EF-1δ cDNA (A) and negative quality control plot (B) for fluorescent quantitative PCR. X axis denotes the Ct value (cycle number of a quantitative PCR reaction), Y axis denotes the ΔRn which is the fluorescence intensity over the background.

fluorescence intensity over the background was 0.999. The Ct value from the negative quality control plot was over 30, indicating that there is no contamination in the FQ-PCR reaction system.

*Overexpression of EF-1δ in 16HBE cells transformed by Cd* The overexpression of human EF-1δ mRNA at different stages of 16HBE cells transformed by $CdCl_2$ was further quantified by FQ-PCR assay (Table 2, Figs. 2 and 3). The human EF-1δ p31 expression at different stages of 16HBE cells transformed by $CdCl_2$ was higher than their corresponding non-transformed cells ($P<0.01$ or $P<0.05$), the overexpression level of human EF-1δ p31 was averagely increased 2.9 folds in Cd-midtransformed cells, 4.3 folds in Cd-transformed cells and 7.2 folds in Cd-tumorigenic cells, indicating that human EF-1δ genes are significantly overexpressed during malignant transformation of 16HBE cells induced by Cd, which is related to the malignancy of cells.

TABLE 2

Overexpression of EF-1δ p31 during Malignant Transformation of 16HBE Cells Induced by $CdCl_2$ (Mean ±SD)

| Cells induced by Cd | n | Human EF-1δ cDNA ($\times 10^6$ copies$^{-1}$ μgRNA | | |
|---|---|---|---|---|
| | | Cell line 1 | Cell line 2 | Cell line 3 |
| Non-transformed Cells | 3 | 1.13 ±0.23 | 1.13 ±0.23 | 1.13 ±0.23 |
| Cd-pretransformed Cells | 3 | 3.79 ±0.17$^{*}$ | 4.59 ±0.49$^{*}$ | 1.55 ±0.13$^{*}$ |
| Cd-transformed Cells | 3 | 5.86 ±0.87$^{**}$ | 7.86 ±0.71$^{**}$ | 3.29 ±0.63$^{*}$ |
| Cd-tumorigenic Cells | 3 | 7.76 ±1.46$^{**}$ | 7.17 ±0.36$^{**}$ | 9.44 ±1.32$^{**}$ |

*Note*. $^{*}P<0.05$, $^{**}P<0.01$ *vs* non-transformed controls (Analysis of variance and Dunnett *t*-test).

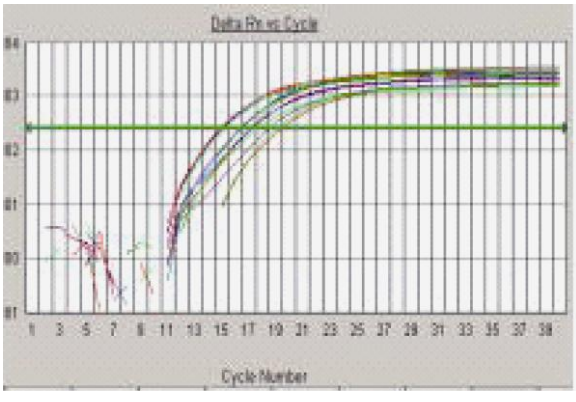


FIG. 2. Representative overexpression of human EF-1δ gene in 16HBE cells induced by $CdCl_2$. X axis denotes the cycle number of quantitative PCR, Y axis denotes the ΔRn which is the fluorescence intensity over the background.

*Sequence Analysis of Overexpressed EF-1δ Gene*

*Confirmation of plasmid pMD18-T-EF-1δ gene*

The inserted plasmid pMD18-T with EF-1δ gene from positive clones was confirmed by restriction enzyme (*Bam*HI, *Eco*RI, and *Hin*dIII) digestion and gel electrophoresis of the digested plasmid. The segments (166 bp and 910 bp) were matched with the expected size of recombinant pMD18-T vector of the EF-1δ gene, indicating that the EF-1δ gene can be inserted into the pMD18-T vector (Fig. 4).

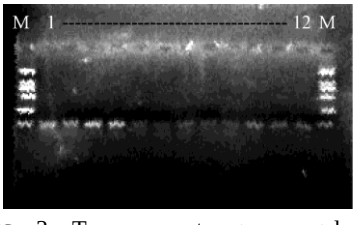


FIG. 3. Two percent agarose gel electrophoresis showing representative human EF-1δ cDNA products. M: 100 bp ladder molecular standard; lanes 1-12: different human EF-1δ cDNA products.

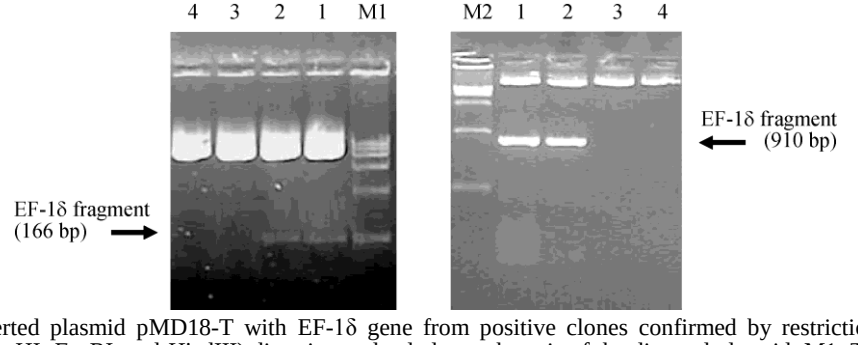


FIG. 4. Inserted plasmid pMD18-T with EF-1δ gene from positive clones confirmed by restriction enzyme (*Bam*HI, *Eco*RI, and *Hin*dIII) digestion and gel electrophoresis of the digested plasmid. M1: TaKaRa DL 2000 (from far to near: 100, 250, 500, 750, 1 000, 2 000 bp); M2: Biochip (from far to near: 100, 1 200, 2 000, 4 000 bp); Lanes 1-2: pMD18-T vector + EF-1δ fragments; Lanes 3-4: pMD18-T vector.

*Sequence of human EF-1δ gene* The overexpressed human EF-1δ genes at different stages of 16HBE cells transformed by $CdCl_2$ were sequenced and compared with those in GenBank. The sequences of target EF-1δ genes and those in GenBank were 100% matched with the basic local alignment search tool (BLAST) (Table 3). Compared with those of control 16HBE cells, no change was observed in the sequence of human EF-1δ cDNA in transformed and tumorigenic cells induced by Cd, indicating that the EF-1δ p31 cDNA region is not a target sequence in carcinogenesis induced by Cd (Fig. 5).

TABLE 3

3 EF-1δ Genes at Different Stages of 16HBE Cells Transformed by Cd with BLAST

| Cells Induced by Cd | Cell Lines | Similarity with BLAST |
|---|---|---|
| Non-transformed Cells | 4 | 100% |
| Cd-pretransformed Cells | 4 | 100% |
| Cd-transformed Cells | 4 | 100% |
| Cd-tumorigenic Cells | 4 | 100% |

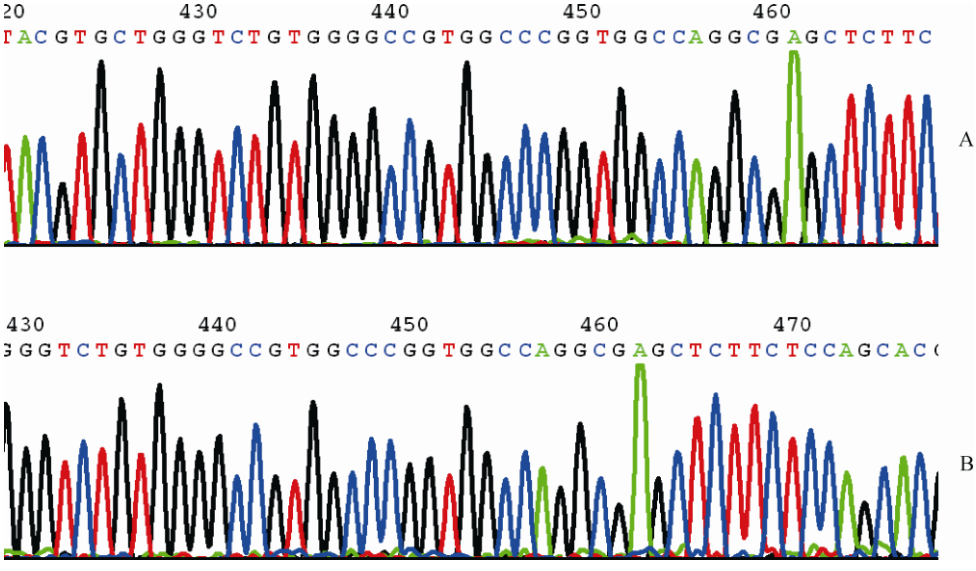


FIG. 5. Sequences of human EF-1δ genes at different stages of 16HBE cells transformed by Cd compared with those in GenBank. A: Sequences of EF-1δ gene from non-transformed cells; B: Sequences of EF-1δ gene from Cd-tumorigenic cells.

## DISCUSSION

Morphological transformation of cells *in vitro* is a phenomenon that has been noted for many years as a complex series of events with patterns of growth control changes similar to carcinogenesis[20-22]. Several studies have shown that rodent cells or human fibroblasts are a sensitive system for cell transformation to chemical agents[23-24]. Recently, Keshava *et al.*[15] have established a model system of morphological cell transformation of BALB/c-3T3 cells using $CdCl_2$. However so far no report is available on malignant transformation of human airway epithelial cells induced by $CdCl_2$. We have used human 16HBE cells as a target of $CdCl_2$ for morphological transformation, and the results demonstrate that malignant transformation in 16HBE cells coupled with soft agar/nude mouse assay is a useful approach to study human carcinogenic potential of Cd[16]. It has been reported that most human tumors originating from epithelial cells and 16HBE cells possess the features of human normal airway epithelial cells[25-26]. Moreover, 16HBE cells are easy to culture and free tumorigenic to nude mice, indicating that 16HBE cell lines can be used as a target for investigation of chemical carcinogens.

Changes, especially genetic changes in expression of genes regulating cell cycle, play an important role in the development of malignant transformation[27-28]. Modifications in the translational machinery of cells, including changes in both eukaryotic translation initiation and elongation

factors, can result in susceptibility to transformation and acquisition of transformed and oncogenic properties in cells[29-30]. For example, enhanced expression of elongation factor-1α (EF-1α) confers susceptibility to carcinogens and UV light-induced transformation to mouse and Syrian hamster cell lines[31]. Furthermore, elevated levels of human EF-1α and EF-1γ are found in tumors of the pancreas, colon, breast, lungs, prostate and stomach relative to normal tissues[32-34]. It is reported that mouse TEF-1δ p31 has been identified as a novel cadmium-responsive proto-oncogene[13-14]. In this study, the expression of human EF-1δ p31 at different stages of 16HBE cells transformed by $CdCl_2$ was higher than that of non-transformed controls, and the expression level of EF-1δ p31 increased with the malignant degrees of cells. No change was found in the sequence of human EF-1δ cDNA in the transformed and tumorigenic cells induced by Cd, indicating that EF-1δ p31 cDNA region is not a target sequence in carcinogenesis induced by Cd and that EF-1δ p31 is significantly overexpressed during transformation of 16HBE cells induced by $CdCl_2$, and overexpression of EF-1δ p31 is positively correlated with malignant transformation of 16HBE cells induced by $CdCl_2$, rather than with DNA mutations. These findings may explain, in part, the cadmium carcinogenesis.

## ACKNOWLEDGEMENTS

The authors thank Dr. Tong-Man ONG for his helpful suggestions, Yuan-Hui HUANG for his pertinent advice on the manuscript, and Dr. Bing LI for his technical assistance.

## REFERENCES

1. Fay R M, Mumtaz M M (1996). Development of a priority list of chemical mixture occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem Toxicol* **34**, 1163-1165.
2. Waalkes M P (2003). Cadmium carcinogenesis. *Mutat Res* **533**(1-2), 107-120.
3. Sorahan T, Esmen, N A (2004). Lung cancer mortality in UK nickel-cadmium battery workers, 1947-2000. *Occup Environ Med* **61**(2), 108-116.
4. Koyu A, Gokcimen A, Ozguner F, *et al*. (2006). Evaluation of the effects of cadmium on rat liver. *Mol Cell Biochem* **20**, 1-5.
5. Nadal M, Schuhmacher M, Domingo J L (2004). Metal pollution of soils and vegetation in an area with petrochemical industry. *Sci Total Environ* **321**(1-3), 59-69.
6. Boguszewska A, Pasternak K (2004). Cadmium--influence on biochemical processes of the human organism. *Ann Univ Mariae Curie Sklodowska* **59**(2), 519-523.
7. Sunderman F M (2001). Nasal toxicity, carcinogenicity, and olfactory uptake of metals. *Ann Clin Lab Sci* **31**, 3-24.
8. Nordberg G, ChinaCad Group (2003). Cadmium and human health: A perspective based on recent studies in China. *J Trace Elem Exp Med* **16**, 307-319.
9. Kriegel A M, Soliman A S, Zhang Q, *et al*. (2006). Serum cadmium levels in pancreatic cancer patients from the East Nile Delta region of Egypt. *Environ Health Perspect* **114**(1), 113-119.
10. Sorahan T (1987). Mortality from lung cancer among a cohort of nickel cadmium battery workers: 1946-84. *Br J Ind Med* **44**, 803-809.
11. Goyer, R A, Liu, J, Waalkes, M P (2004). Cadmium and cancer of prostate and testis. *Biometals* **17**(5), 555-558.
12. IARC (1993). Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, Vol. **58**, 119-238, International Agency for Research on Cancer, Lyon, France.
13. Joseph P, Lei, Y X, Whong, W Z, *et al*. (2002). Oncogenic potential of mouse translation elongation factor-1 delta, a novel cadmium-responsive proto-oncogene. *J Biol Chem* **277**(8), 6131-6136.
14. Lei Y X, Chen J K, Wu Z L (2002). Blocking the translation elongation factor-1δ with its antisense mRNA results in a significant reversal of its oncogenic potential. *Teratog Carcinog Mutagen* **22**(5), 377-383.
15. Keshava N, Zhou G, Hubbs A F, *et al*. (2000). Transforming and carcinogenic potential of cadmium chloride in BALB/c-3T3 cells. *Mutat Res* **448**(1), 23-28.
16. Lei Y X, Wei L, Wang M, *et al*. (2008). Malignant transformation and abnormal expression of eukaryotic initiation factor during human bronchial epithelial cells induced by cadmium chloride. *Biomed Environ Sci* **21**(4), 332-338.
17. Cozens A L, Yezzi M J, Kunzelmann K, *et al*. (1994). CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* **10**(1), 38-47.
18. Dunkel, V C, Rogers C, Swiernga S H, *et al*. (1991). Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: III. Cell transformation in C3H/10T1/2 mouse embryo cell, BALB/c-3T3 mouse fibroblast and Syrian hamster embryo cell cultures. *Mutat Res* **246**, 285-300.
19. Gelmini S, Orlando C, Sestini R, *et al*. (1997). Quantitative polymerase chain reaction-based homogeneous assay with fluorogenic probes to measure c-erbB-2 oncogene amplification. *Clin Chem* **43**(5), 752-758.
20. Zhang, Z R (2004). Cultural cytology and technique for cell culture. pp 129-133, Shanghai Science & Technology Press.
21. Berwald Y, Sachs L. (1963). *In vitro* transformation with chemical carcinogens. *Nature* **200**, 1182-1184.
22. Berwald Y, Sachs L. (1965). *In vitro* transformation of normal cells to tumor cells by carcinogenic hydrocarbons. *J Natl Cancer Inst* **35**, 641-661.
23. Saffiotti U, Bertolero F (1989). Neoplastic transformation of BALB/3T3 cells by metals and quest for induction of a metastatic phenotype. *Biol Trace Elem Res* **21**, 475-482.
24. Landolph J R (1985). Mechanisms of chemically induced multistep neoplastic transformation in C3H10T1/2 cells. *Carcinogenesis* **10**, 211-223.
25. Cairns J (1975). Mutation selection and the natural history of cancer. *Nature* **255**, 197-200.
26. Cruenert D C, Finkbeiner W E, Widdicombe J H (1995). Culture and transformation of human airway epithelial cells. *Am J Physiol* **268**, 1347-1360.
27. Vogelstein B, Kinzler R W (1993). The mulistep nature of cancer. *Trends Genet* **9**, 138-141.
28. Zimmer S G, Debenedetti A, Graff J R. (2000). Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor promotion, growth, invasion and metastasis. *Anticancer Res* **20**, 1343-1352.
29. Riis B, Rattan S I S, Clark B F C, *et al*. (1990). Eukaryotic translation elongation factiors. *TIBS* **15**, 420-424.
30. Sonenbeerg N. (1993). Translation factors as effectors of cell growth and tumorigenesis. *Curr Opin Cell Biol* **5**, 955-960.

31. Tatsuka M, Mitsui M, Wada A, *et al*. (1992). Elongation factor-1 alpha gene determines susceptibility to transformation. *Nature* **359**, 333-336.
32. Grant A G, Flomen R M, Tizard M L V, *et al*. (1992). Differential screening of human pancreatic adenocarcinoma lambda gt11 expression library has identified increased transcription of elongation factor EF-1 alpha in tumor cells. *Int J Cancer* **51**, 740-745.
33. Chi K, Jones D V, Frazier M L (1992). Expression of an elongation factor 1 gamma-related, equence in adenocarcinomas of the colon. *Gastroenterology* **103**, 98-102.
34. Su Z, Goldstein N I, Fisher P B (1998). Antisense inhibition of the PTI-1 oncogene reverses cancer phenotypes. *Proc Natl Acad Sci UAS* **95**, 1764-1769.

(*Received March* 4, 2009 *Accepted March* 7, 2010)