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# Up-regulation of expression of translation factors – a novel molecular mechanism for cadmium carcinogenesis

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## Abstract

The molecular mechanisms potentially responsible for cadmium carcinogenesis were investigated by differential gene expression analysis of Balb/c-3T3 cells morphologically transformed with cadmium chloride. Differential display analysis of gene expression revealed overexpression of mouse Translation Initiation Factor 3 (TIF3; GenBank Accession Number AF 271072) and Translation Elongation Factor-1δ (TEF-1δ; GenBank Accession Number AF 304351) in the transformed cells compared with the control cells. The full length cDNAs for TIF3 and TEF-1δ were cloned and sequenced. Transfection of mammalian cells with an expression vector containing either TIF3 or TEF-1δ cDNA resulted in overexpression of the encoded protein. Overexpression of the cDNA-encoded TIF3 and TEF-1δ proteins in NIH3T3 cells was oncogenic as evidenced by the appearance of transformed foci capable of anchorage-independent growth on soft agar and tumorigenesis in nude mouse. Blocking the translation of TIF3 and TEF-1δ proteins using the corresponding antisense mRNA resulted in a significant reversal of the oncogenic potential of cadmium transformed Balb/c-3T3 cells as evidenced from the suppression of anchorage-independent growth on soft agar and diminished tumorigenesis in nude mouse. These findings demonstrate that the up-regulation of expression of TIF3 and TEF-1δ is a novel molecular mechanism responsible, at least in part, for cadmium carcinogenesis. (Mol Cell Biochem **255**: 93–101, 2004)

*Key words*: cadmium, carcinogenesis, mechanisms, gene expression, translation factors

## Introduction

Cadmium (Cd) is a toxic transition metal of continuing occupational and environmental concern. It has a variety of industrial uses such as smelting, electroplating, welding, manufacture of Cd alloys, and production of nickel-cadmium batteries [1]. The increased industrial usage of Cd has caused an increase in Cd production and a concomitant rise in contamination of soil, water, and atmosphere resulting in significant human exposure to the metal. It has been estimated that each year more than half a million people are occupationally exposed to Cd in the US alone [2]. In addition, the general population is at risk of exposure to Cd through ingestion of contaminated food and water, inhalation of contaminated air, and through exposure to cigarette smoke.

There have been several reports in the past documenting the toxicological responses of humans and experimental animals to the exposure to Cd; and they include kidney damage, respiratory diseases, and neurologic disorders [1, 3, 4]. Of particular importance among the toxic effects of Cd is its convincing and well-documented role as a human carcinogen. The International Agency for Research on Cancer [1] has concluded, based on all available epidemiological and experimental data, that there is adequate evidence to classify Cd as a type I human carcinogen. Despite the strong evidence supporting the carcinogenic potential of Cd, the underlying mechanisms responsible for its carcinogenic action are not clearly understood. The almost negligible ability of Cd to cause direct mutagenic DNA damage or adducts [5] seems to suggest that non-genotoxic or epigenetic mechanism(s)

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may play an important role in the carcinogenicity of Cd. Recent developments in toxicogenomics have demonstrated the potential of studies investigating differential gene expression profiles to elucidate the cellular/molecular mechanisms of chemical carcinogenesis. Therefore, by employing differential display analysis of gene expression in Balb/c-3T3 cells transformed with cadmium, we have investigated the potential mechanisms of Cd-induced cell transformation and tumorigenesis. The results obtained from our studies have identified an up-regulation in the expression of Translation Initiation Factor 3 (TIF3, GenBank accession number AF 304351) and Translation Elongation Factor-1δ (TEF-1δ, GenBank accession number AF 271072) as a novel molecular mechanism for Cd carcinogenesis [6–8]. The potential implications of these novel findings are reviewed in light of the current knowledge regarding the mechanisms of Cd carcinogenesis.

## Identification and cDNA cloning of Cd-responsive translation factors

Cell transformation coupled with the tumorigenesis assay using immune-deficient mice exhibits characteristics analogous to *in vivo* carcinogenesis and therefore has long been used as a surrogate to study the carcinogenic potential of chemicals [9]. Exposure of contact-inhibited Balb/c-3T3 cells to 6–12 μM $CdCl_2$ for 72 h resulted in cell transformation as evidenced by the appearance of morphologically transformed foci capable to grow subcutaneously as tumors in athymic nude mice [10]. Total RNA, free of any contaminating DNA, isolated from control and Cd-transformed Balb/c-3T3 cells were analyzed for differential gene expression by restriction fragment differential display-polymerase chain reaction (RFDD-PCR). The RFDD-PCR was done using the Display Profile™ kit (Display Systems Biotech, Vista, CA, USA) according to the manufacturer's instructions. Stated briefly, double stranded cDNA synthesized from RNA isolated from control and transformed cells were PCR-amplified using Cy5-labeled 5′-primer in combination with each of the 64 display probe primers of the kit, resulting in the amplification of ~ 25,000 distinct cDNA fragments (Display Systems Biotech, Vista, CA, USA). The PCR amplified fragments were size fractionated by 6% denaturing polyacrylamide gel electrophoresis and detected by scanning the gel using a Storm Fluorimager (Molecular Dynamics Inc., Sunnyvale, CA, USA). Several cDNA fragments were found to be differentially expressed in the Cd-transformed cells compared with the control cells (Fig. 1A). The differentially expressed cDNA fragments were PCR-reamplified using the original set of primers and were sub-cloned into the TA-cloning vector (Invitrogen, Carlsbad, CA, USA). The cDNA fragments were sequenced using an ABI PRISM automated DNA sequencer (Perkin Elmer-Life Sciences, Wellesley, MA, USA) and identified by comparing their nucleotide sequence to those available in the GenBank data base by the BLAST analysis.

Two of the differentially expressed cDNA fragments, designated DD51 (294-bp) and DD47 (125-bp), exhibited significant sequence similarity to human eukaryotic initiation factor 3 (also known as TGF-β receptor interacting protein II, GenBank accession number U39067) and translation elongation factor-1δ (GenBank accession number Z21507), respectively. The differentially expressed cDNA fragments were therefore identified as mouse Translation Initiation Factor 3 (TIF3, GenBank accession number AF 271072) and Translation Elongation Factor-1δ (TEF-1δ, GenBank accession number AF 304351). Northern hybridization of TIF3 and TEF-1δ cDNA fragments to total RNA isolated from the control and Cd-transformed Balb/c-3T3 cells detected transcript sizes of approximately 1.1 kb each (Fig. 1B). Results of Northern hybridization, furthermore, confirmed the overexpression of both TIF3 and TEF-1δ in the Cd-transformed cell lines compared to that of the control.

The full-length cDNA for both TIF3 and TEF-1δ were cloned by the RACE technique using the SMART-RACE cDNA amplification kit (Clontech Laboratories, Palo Alto, CA) following the procedures provided by the manufacturer. The full-length cDNAs were cloned initially as 5′- and 3′-RACE fragments and they were subsequently ligated to ob-

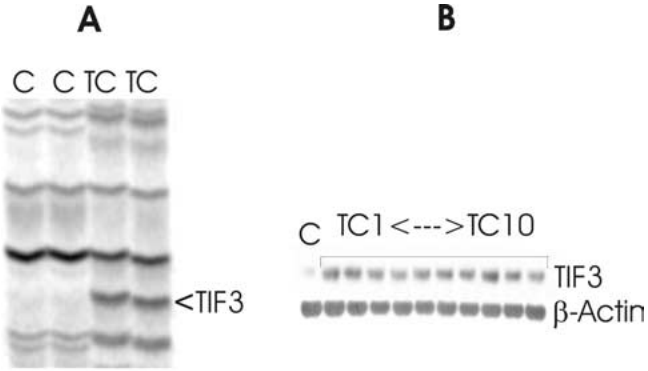


*Fig. 1.* Overexpression of translation initiation factor 3 (TIF3) in Balb/c-3T3 cells transformed with CdCl2. Total RNA isolated from the control and $CdCl_2$-transformed BALB/c-3T3 cells were analyzed for differential gene expression by DDRT-PCR as described in the text. (A) Differential display analysis revealed the overexpression of a 294-bp cDNA fragment in the transformed cells that was identified as TIF3 (C – control cells; TC – transformed cells). (B) Northern blot analysis of RNA isolated from control [C] and ten different transformed cell lines [TC1<-->TC10] using TIF3 cDNA fragment as the probe confirmed overexpression of the TIF3 transcript in the $CdCl_2$-transformed cells. Similar overexpression was noticed for mouse translation elongation factor-1δ (TEF-1δ) in the transformed cells compared with the non-transformed control cells (data not presented). Data reproduced with copyright permissions from [6, 7].

tain the full-length cDNAs. The full-length TIF3 and TEF-1δ cDNAs consisted of 1097 and 1004 nucleotides, respectively. Analysis of the nucleotide sequence revealed open reading frames encoding 325 and 281 amino acids for TIF3 and TEF-1δ, respectively (the nucleotide and predicted amino acid sequences for TIF3 and TEF-1δ are available in the GenBank under accession numbers AF 304351 and AF 271072, respectively).

## Overexpression of TIF3 and TEF-1δ results in cell transformation

The overexpression of TIF3 and TEF-1δ observed in the Cd-transformed cells raised an important question regarding the significance of overexpression of the translation factors – whether the overexpression of these factors is oncogenic and responsible for the Cd-induced cell transformation or whether their overexpression is simply a marker for the transformed nature of the cells? As a prelude to addressing this question, we first expressed the cDNA encoded proteins in mammalian cells by transfection. For this, the open reading frames of TIF3 and TEF-1δ were fused in frame with the V5 epitope and 6X His tag of the expression vector, pcDNA3.1D/V5-His-TOPO (Invitrogen, Carlsbad, CA, USA). The nucleotide sequence, 5′-CACC-3′, was added 5′- to the start codon of the respective cDNA and the stop codon was removed. Plasmid DNA prepared using Qiagen maxi preparation kit (Qiagen, Valencia, CA, USA) was used to transfect Chinese hamster ovary (CHO) and monkey kidney COS7 (COS7) cells by the calcium phosphate transfection procedure (Clontech Laboratories, Palo Alto, CA, USA). Cell Lysates prepared from the transfected cells were analyzed for transfection-mediated overexpression of the fusion protein by Western blot analysis, using an antibody for the V5 epitope of the fusion protein and detected with an enhanced chemiluminescent kit (Amersham-Pharmacia Biotech, Piscataway, NJ, USA). Transfection of COS7 and CHO cells resulted in expression of the cDNA-encoded TIF3 and TEF-1δ proteins with approximate molecular weights of 35- and 31-kDa, respectively (Fig. 2).

To test whether the overexpression of TIF3 and TEF-1δ was oncogenic, so as to result in cell transformation, NIH3T3 cells were transfected with the expression plasmid for the corresponding cDNAs. NIH3T3 cells are routinely used and have been well characterized with respect to their ability to identify cellular proto-oncogenes [11, 12]. Transfection of NIH3T3 cells with the pcDNA3.1 expression plasmids for TIF3 and TEF-1δ resulted in the overexpression of the encoded proteins leading to cell transformation, as evidenced from the appearance of transformed foci (Figs 3A and 3B). The transformed nature of the foci was also confirmed by the anchorage-independent growth potential of the cells derived


*Fig. 2.* Transfection and expression of mouse translation initiation factor 3 (TIF3) cDNA in mammalian cells. Chinese hamster ovary cells were transfected either with the vector – pcDNA3.1D/V5-His-TOPO alone (+Vector) or with the vector containing the entire open reading frame of TIF3 cDNA (+TIF3) as described in the text. Lysates prepared from the cells were analyzed for expression of the fusion protein by Western blotting using antibody for the V5 epitope. The actual size of the fusion protein cross-reacting with the antibody is approximately 39 kDa of which 3.6 kDa corresponds to the V5 epitope and the polyhistidine region derived from the vector. TIF3 [4], TIF3 [13] and TIF3 [17] are three different cell lines permanently expressing the TIF3-V5 epitope fusion protein. Similar results were obtained when CHO cells were transfected with translation elongation factor-1δ cDNA (data not shown). Data reproduced with copyright permissions from [6, 7].

from the foci on soft agar (Fig. 3C). The transformed phenotypes of the NIH3T3 cells overexpressing cDNA-encoded TIF3 and TEF-1δ were further confirmed by determining the tumorigenic potential of these cells in nude mice. For this purpose, immune-deficient, athymic nude mice (Harlan Sprague Dawley, Indianapolis, IN, USA) were injected subcutaneously (s.c.) with control and transformed NIH3T3 cells ($2 \times 10^6$ cells/mouse), and the mice were observed for the appearance of tumors for a maximum period of 2 months. Overexpression of TIF3 and TEF-1δ proteins exhibited tumorigenic potential as evidenced by their development as s.c. tumors in the nude mice (Fig. 3D). The control cells, on the other hand, failed to develop into tumors when injected in the nude mice.

## Reversal of cadmium carcinogenesis by antisense TIF3 and TEF-1δ mRNA

As described above, both TIF3 and TEF-1δ were overexpressed in Cd-transformed Balb/c-3T3 cells and the transfection-mediated overexpression of these translation factors was onco-

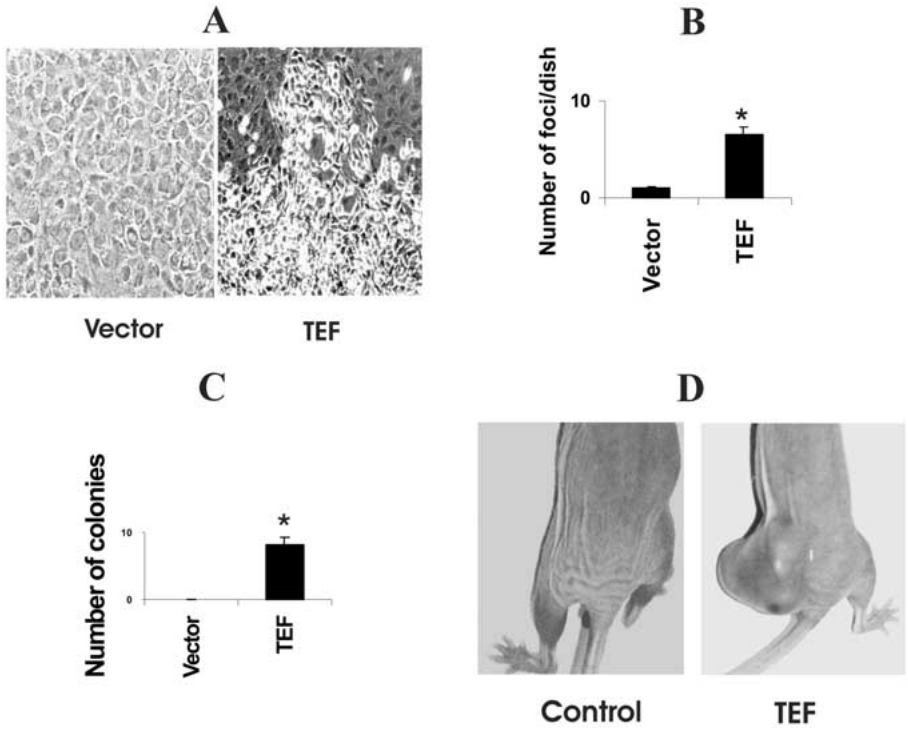


*Fig. 3*. Overexpression of translation elongation factor-1δ (TEF-1δ) results in cell transformation and tumorigenesis. NIH3T3 cells exhibiting contact inhibition were transfected with pcDNA 3. 1D/V5-His-TOPO alone (Vector) or with the vector containing the TEF-1δ cDNA (TEF) as described in the text. Transfection-mediated overexpression of the TEF-1δ protein resulted in transformation of NIH3T3 cells as evidenced by the appearance of transformed foci, anchorage-independent growth of the transformed cells on soft agar, and as subcutaneous tumors in nude mice. (A) Photomicrograph of cells transfected either with the vector or with the TEF-1δ plasmid DNA; (B) Number of transformed foci in cells transfected either with the vector or with the TEF-1δ plasmid DNA; (C) Anchorage independent growth of the NIH3T3 cells transformed by transfection-mediated overexpression of TEF-1δ; (D) Tumorigenic potential of NIH3T3 cells transfected either with the vector or with the TEF-1δ plasmid DNA. * Statistically significant compared with the control ($p < 0.05$), where $n = 4$. Similar results were obtained with translation initiation factor 3 (TIF3) cDNA (data not presented). Data reproduced with copyright permissions from [6, 7].

genic. Obviously, it was logical to investigate whether transformation of the Balb/c-3T3 cells by exposure to Cd was mediated through the overexpression of TIF3 and TEF-1δ. The direct role of the TIF3 and TEF-1δ genes in the Cd-induced cell transformation and tumorigenesis was evaluated by blocking the translation of TIF3 and TEF-1δ in the Cd-transformed Balb/c-3T3 cells by employing corresponding antisense mRNA and then testing the antisense-inhibited cells for their oncogenic potential. The antisense TIF3 and TEF-1δ plasmids were constructed by sub-cloning the corresponding cDNA in the reverse (3′-5′) orientation within the pcDNA3.1/V5-His-TOPO expression vector. The orientation of the cDNA within the resulting plasmid was confirmed by restriction enzyme digestion and agarose gel electrophoresis analysis of the antisense plasmid DNA. Cadmium transformed Balb/c-3T3 cells overexpressing TIF3 and TEF-1δ genes were transfected either with TIF3 or TEF-1δ antisense plasmid by the calcium phosphate transfection protocol (Clontech Laboratories, Palo Alto, CA, USA). Stable transfectants were selected using G418 at a concentration of 400 μg/ml – a dose that results in the death of all of the untransfected cells. The resulting G418-selected transfectants were then screened for the expression of TIF3 and TEF-1δ antisense mRNA by RT-PCR. PCR primers specific for each antisense mRNA, one immediately downstream to the transcription start site of the vector and the other one specific either for TIF3 or TEF-1δ mRNA in the antisense orientation were used in the RT-PCR to amplify the respective antisense mRNA. The stable transfectants overexpressing either TIF3 or TEF-1δ antisense mRNA were then analyzed for their oncogenic potential based on their ability to grow either as anchorage-independent colonies on soft agar or as subcutaneous tumors in nude mice by methods as described above.

Transfection of the Cd-transformed Balb/c-3T3 cells with the antisense plasmid DNA provided the cells with protection from the cytotoxic effects of G418. The expression of TIF3 and TEF-1δ antisense mRNA in the stable transfectants was further confirmed by the PCR amplification of the antisense mRNA that was detectable only in the cells transfected with the corresponding antisense plasmids [6, 7]. Blocking

the translation of TIF3 or TEF-1δ in the Cd-transformed Balb/c-3T3 cells by the respective antisense mRNA resulted in a significant reversal of the oncogenic potential of the Cd-transformed Balb/c-3T3 cells as evidenced from a decrease in the number of anchorage-independent colonies growing on soft agar (Fig. 4A) and the reduced tumorigenic potential of the antisense mRNA-inhibited cells in nude mice (Fig. 4B) compared with the corresponding controls overexpressing TIF3 and TEF-1δ.

## Conclusions and perspectives

Despite being categorized as a type I human carcinogen, the underlying mechanisms responsible for the carcinogenic potential of Cd are rather poorly understood. The almost negligible ability of Cd to act as a direct carcinogen, or lack there of, has prompted investigators to propose alternate, indirect or epigenetic mechanisms for Cd carcinogenesis. The potential of Cd to deregulate the expression of genes, especially those controlling cell growth and division, has been demonstrated independently by several investigators and appears to be a plausible mechanism responsible for Cd carcinogenesis. Accordingly, exposure to Cd results in the deregulation of expression of early response genes, c-*fos*, c-*jun*, c-*myc*, etc. [13, 14]; cytoprotective genes such as metallothionein [15]; heat shock proteins [13, 14]; genes involved in glutathione synthesis and function [18, 19]; genes responsible for protection against oxidative stress [14, 17]; genes encoding for transcription factors [21–24]; and several other unclassified genes [25–27]. These reports, based on the recognized functions of the deregulated genes, seem to indicate that alterations in gene expression provide a possible alternative mechanism for Cd carcinogenesis. However, they have not conclusively linked the Cd-induced alterations in gene expression to the carcinogenic action of the metal. Based on the results of a series of comprehensive studies, we have obtained significant evidence to conclude that the Cd-induced cell transformation and tumorigenesis are mediated, at least in part, through the overexpression TIF3 and TEF-1δ. The cardinal findings that support such a conclusion are: (i) exposure of Balb/c-3T3 cells to $CdCl_2$ resulted in cell transformation and the transformed cells exhibited tumorigenic potential in athymic nude mice [10]; (ii) TIF3 and TEF-1δ were found consistently and reproducibly overexpressed in all (10 out of 10) $CdCl_2$-transformed Balb/c-3T3 cell lines that were analyzed; (iii) transfection-mediated overexpression of TIF3 and TEF-1δ cDNA-encoded proteins in NIH3T3 cells resulted in oncogenesis, as evidenced by the anchorage-independent growth potential of the transformed cells on soft agar as well as their growth as subcutaneous tumors in nude mice; and iv) blocking the translation of overexpressed TIF3 and TEF-1δ in the Cd-transformed Balb/c-3T3 cells by the corresponding antisense mRNA resulted in a significant reversal of the oncogenic potential of the transformed cells. This was evidenced from a significant reduction in the growth potential of the antisense inhibited cells on soft agar as well as from their reduced growth as subcutaneous tumors in nude mice compared to the corresponding control cells.

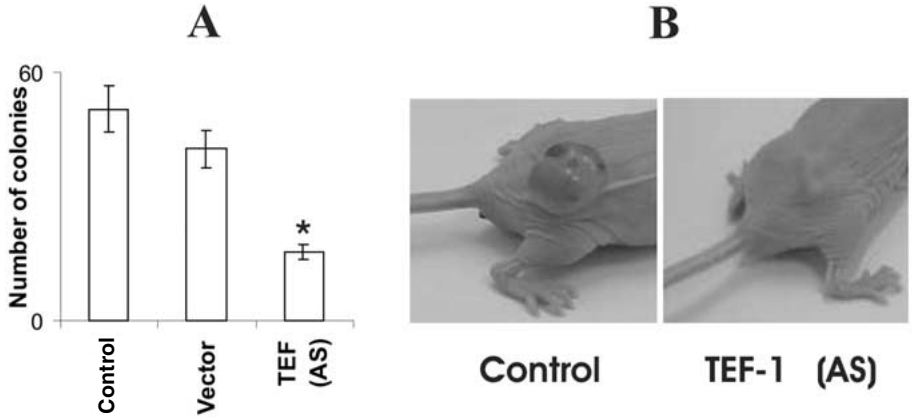


*Fig. 4.* Reversal of cadmium carcinogenesis by antisense translation elongation factor-1δ (TEF-1δ) mRNA. BALB/c-3T3 cells, transformed with $CdCl_2$, were transfected with the pcDNA3.1 vector alone (Vector) or with the vector expressing the antisense TEF-1δ mRNA [TEF (AS)]. Stable transfectants were selected using G418 (400 μg/ml). (A) The cells were tested for their capacity to grow as anchorage-independent colonies on soft agar. The colonies were stained with *p*-iodonitrotetrazolium violet (Sigma Chemical Co., St. Louis, MO, USA) and colonies larger than 0.1 mm were quantitated. (B) The stable transfectants were tested for their tumorigenic potential using nude mice. For comparison, mice injected with cells transfected with the vector alone and the TEF-1δ (AS) plasmid are shown; Similar results were obtained with translation initiation factor 3 (TIF3) cDNA (data not presented). *Statistically significant ($p < 0.05$), where $n = 4$. Data reproduced with copyright permissions from [6, 7].

The complex, multi-step process of translation in eukaryotes is regulated by the expression of several genes that are designated as translation (initiation, elongation, and termination) factors. There have been several reports suggesting that the modification of translation regulation of cells due to alterations in the expression and/or function of translation factors may result in the development of oncogenic properties by cells [28–31]. Enhanced expression of specific translation factors, for example, has been shown to confer susceptibility to carcinogen and UV-induced cell transformation [32]. Further evidence supporting a role for translation factors in cancer is derived from the observation that the cellular concentrations of several of these factors are significantly higher in tumor cell lines and in tumors originated from multiple tissues compared with the corresponding controls [12, 33–43]. Although, not fully understood, the observed involvement of translation factors in cell growth, division, and senescence further support a potential role for translation factors in carcinogenesis. In *Saccharomyces cerevisiae*, mutations of EF-1α lead to the loss of proofreading potential, resulting in translational infidelity [44, 45] – a hallmark of carcinogenesis [46]. The cellular concentration and catalytic activity of EF-1α is reported to be lower during senescence in *Drosophila melanogaster* and its forced expression results in cell proliferation and extension of life span [47, 48]. Translation factors have also been found to play an active role in mitosis through their association with the mitotic spindle apparatus [49]. Overexpression of translation factors may also confer selective translational up-regulation of certain genes that are involved in cell growth and division so as to result in carcinogenesis. This is best illustrated by the translational up-regulation of genes such as cyclin D1 [50, 51] and c-*myc* [53, 54] in cells that exhibit overexpression of eIF4E – the most investigated oncogenic translation factor [42, 55].

Elucidation of the aforementioned novel mechanism for Cd carcinogenesis, involving the overexpression of TIF3 and TEF-1δ, has raised several interesting questions regarding the role of these and other translation factors in carcinogenesis, including those due to exposure to metals and other chemical carcinogens. The important issues that deserve immediate and further attention are the following:

(a) *TIF3 and TEF-1δ overexpression – a mechanism specific for Cd carcinogenesis?* Even though the results presented in this communication have conclusively demonstrated that the overexpression of TIF3 and TEF-1 is a novel mechanism for Cd carcinogenesis, it is not known whether the same is true with respect to carcinogenesis due to other metals and non-metallic chemical carcinogens.

(b) *Involvement of other translation factors in Cd carcinogenesis?* Besides TIF3 and TEF-1δ, there are an estimated 3 dozen translation factors that have been identified and characterized with respect to their role in protein synthesis. It is therefore important to investigate whether the deregulation of expression of translation factors other than TIF3 and TEF-1δ are involved in carcinogenesis due to exposure to Cd and other chemicals. This is particularly important since the oncogenic potential of a few other translation factors has been reported, even though their involvement in chemical carcinogenesis has not been studied [12, 41–43, 56–59].

(c) *Mechanism(s) of Cd-induced overexpression of TIF3 and TEF-1δ.* Despite the demonstration that both TIF3 and TEF-1δ are overexpressed in Balb/c-3T3 cells morphologically transformed with $CdCl_2$ compared with the control, the underlying mechanism(s) responsible for their overexpression is not known. Exposure of cells to Cd is known to result in alterations in gene expression and several mechanisms including perturbations in the cellular concentration of secondary messengers such as $Ca^{2+}$ and reactive oxygen species [14, 60], changes in cellular phosphorylation status [23, 61], and activation of transcription factors [21–24] have all been attributed to be responsible for the Cd-induced alterations in gene expression. Whether and how any of these mechanisms are involved in the Cd-induced overexpression of TIF3 and TEF-1δ has not yet been determined. Furthermore, cloning and analysis of the promoters of TIF3 and TEF-1δ may reveal the involvement of specific regulatory elements and mechanisms responsible for the Cd-induced overexpression of these genes.

(d) *Mechanisms of cell transformation by TIF3 and TEF-1δ overexpression.* No information is currently available to explain the mechanism(s) of cell transformation brought about by the overexpression of TIF3 and TEF-1δ. The observation that the Cd-induced overexpression of TIF3 and TEF-1δ results in loss of cell growth control culminating in cell transformation may suggest that these factors can function as members of the cellular signal transduction network responsible for cell growth control. Identification of the members of the cellular signal transduction pathways that are influenced by the overexpression of TIF3 and TEF-1δ and elucidation of their modes of action are, therefore, expected to reveal the mechanisms of cell transformation caused by the Cd-responsive translation factors. It is furthermore possible that the overexpression of TIF3 and TEF-1δ might have resulted in translational up-regulation of specific genes facilitating a selective growth advantage to cells; and this, in turn, results in their transformation and tumorigenesis. Such a hypothesis is supported by the observation that the overexpression of the translation factor eIF4E is oncogenic and is often associated with translational up-regulation of cyclin D1 [50, 51], *ras* [52, 62], c-*myc* [53, 54], and ornithine decarboxylase [63] – genes that are known to play a significant role in cell transformation and carcinogenesis.

(e) *Use of alterations in expression of translation factors as a molecular signature for chemical carcinogenesis.* Data reviewed in this article has demonstrated a definite relationship between the cellular expression level of the Cd-responsive translation factors, TIF3 and TEF-1δ, and the transformed phenotype of the cells. Similarly, overexpression of a few other translation factors has been reported in human tumor cell lines and in tumor samples of multiple tissue origin [12, 33–43]. The absolute requirement for the elevated cellular expression levels of the oncogenic translation factors in order to maintain the transformed phenotype of the cells was evidenced from the reversal of the oncogenic properties of the cells in which the overexpressed translation factors were specifically inhibited by employing corresponding antisense mRNA [6–8, 64]. It may, therefore, be possible to use the cellular expression levels of the oncogenic translation factors as markers of carcinogenesis, including those due to occupational exposure to chemical carcinogens. Of course, such an approach will require further validation of the translation factors with respect to their role in cancer development. Once validated appropriately, the cellular levels of the oncogenic translation factors may be used as diagnostic and prognostic markers of carcinogenesis. They may also be used as potential indicators in risk assessment under conditions of occupational exposure.

In conclusion, the data demonstrating the overexpression of TIF3 and TEF-1δ in the Cd-transformed Balb/c-3T3 cells represents a major advance in understanding the molecular mechanisms of Cd carcinogenesis. As discussed above, much more needs to be understood regarding the roles of TIF3 and TEF-1δ, and perhaps of other translation factors, in carcinogenesis including carcinogenesis due to exposure to chemicals such as those found in occupational environments. Once fully understood, it may be possible to use the expression levels of translation factors as marker(s) for carcinogenesis, thus facilitating the identification of individuals who are exposed to potential chemical carcinogens, and therefore are at a higher risk to develop cancer. Alternatively, therapeutic strategies targeting the abnormally expressed translation factors may be developed to treat cancer due to occupational exposure to chemical carcinogens.

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