

Oncogenic Potential of Mouse Translation Elongation Factor-1 δ , a Novel Cadmium-responsive Proto-oncogene*

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Pius Joseph‡, Yi-Xiong Lei, Wen-Zong Whong, and Tong-Man Ong

From the Molecular Epidemiology Laboratory, Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505

The molecular mechanisms potentially responsible for cadmium-induced cell transformation and tumorigenesis were investigated using Balb/c-3T3 cells transformed with cadmium chloride. Differential display analysis of gene expression revealed consistent and reproducible overexpression of a transcript in the transformed cells compared with the nontransformed cells. The full-length cDNA corresponding to the differentially expressed transcript was cloned and was identified as mouse translation elongation factor-1 δ subunit (TEF-1 δ ; GenBank™ accession number AF304351). Nucleotide sequence analysis of TEF-1 δ cDNA revealed an open reading frame encoding the predicted protein of 281 amino acids and exhibited significant conservation with the corresponding protein of human, *Xenopus laevis*, and *Artemia*. The presence of a leucine zipper motif, characteristic of translation elongation factor-1 δ , was also found in the mouse TEF-1 δ . A 31-kDa protein was detected in eukaryotic cells transfected with an expression vector containing the TEF-1 δ cDNA. Overexpression of the TEF-1 δ protein by transfection was oncogenic in NIH3T3 cells as evidenced by the appearance of transformed foci exhibiting anchorage-independent growth and the potential to grow as tumors in nude mice. Blocking the translation of TEF-1 δ with antisense TEF-1 δ mRNA resulted in a significant reversal of the oncogenic potential of cadmium-transformed Balb/c-3T3 cells as evidenced from suppression in anchorage-independent growth and tumorigenesis in nude mice. Our findings demonstrate, for the first time, that the cell transformation and tumorigenesis induced by cadmium are due, at least in part, to the overexpression of TEF-1 δ , a novel cadmium-responsive proto-oncogene.

Cadmium is ranked seventh on the “Top 20 Hazardous Substances Priority List” by the Agency for Toxic Substances and Disease Registry and the United States Environmental Protection Agency (1). Significant quantities of cadmium compounds are used in various industries (2), and a large number of workers are potentially exposed to toxic levels of the metal (3, 4). Furthermore, large quantities of this highly toxic metal are detected in the atmosphere, as well as in food and water, that result in nonoccupational exposure in humans (3, 5). Cadmium

compounds have been detected in significant quantities in the tissues and various organs of individuals exposed to the metal, and the toxicological responses of exposure to cadmium include kidney damage, respiratory diseases, and neurologic disorders (3, 6).

Cadmium induces kidney, prostate, and testicular cancer in rats and mice (3). An increased risk for lung cancer has been reported in people exposed to cadmium (7, 8). The International Agency for Research on Cancer has classified cadmium as a type I human carcinogen based on experimental and epidemiological evidence documenting its carcinogenic potential (9, 10). However, the underlying molecular mechanisms responsible for cadmium-induced malignant transformation are not known. We are conducting studies to test the hypothesis that cadmium-induced deregulation of expression of cancer-related genes may account for the cell transformation and carcinogenesis induced by this industrially and environmentally important metal. Recently, we have reported that the proto-oncogenes, *c-fos*, *c-jun*, and *c-myc*, are overexpressed in Balb/c-3T3 cells morphologically transformed with CdCl₂ (11). In the present study, we employed differential display analysis of gene expression to investigate the involvement of novel cadmium-responsive genes in the cell transformation and tumorigenesis brought about by this carcinogenic metal. In this paper, we document that the mouse translation elongation factor-1 δ subunit (TEF-1 δ)¹ is overexpressed in Balb/c-3T3 cells morphologically transformed with CdCl₂. Furthermore, we provide evidence for the oncogenic potential of TEF-1 δ by its transfection-mediated overexpression, resulting in transformation of NIH3T3 cells and tumorigenesis in nude mice. The oncogenic potential of the CdCl₂ transformed Balb/c-3T3 cells was reversed significantly by expression of the antisense TEF-1 δ mRNA. These results provide definitive evidence that TEF-1 δ is a novel cadmium-responsive cellular proto-oncogene.

EXPERIMENTAL PROCEDURES

Cell Culture, Isolation of RNA, and mRNA Differential Display—Morphological transformation of contact inhibited Balb/c-3T3 cells with CdCl₂ and the development of cell lines from the transformed foci were done as previously reported from our laboratory (12). Briefly, early passages of Balb/c-3T3 cells exhibiting contact inhibition were treated with 6–12 μ M CdCl₂ for 72 h, and the transformed foci were isolated. Cell lines derived from these foci were confirmed for their tumorigenic potential in nude mouse and were grown in minimum essential medium (Sigma) containing 7.5% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Total RNA, free from DNA, was isolated from the nontransformed cells, spontaneously transformed cells, and the CdCl₂-transformed cells using the RNeasy Mini

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF304351.

‡ To whom correspondence should be addressed: MS 3014, Toxicology and Molecular Biology Branch, CDC/NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505. Tel.: 304-285-6240; Fax: 304-285-5708; E-mail: pcj5@cdc.gov.

¹ The abbreviations used are: TEF-1 δ , the δ subunit of mouse translation elongation factor-1; RFDD-PCR, restriction fragment differential display-polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; RACE, rapid amplification of cDNA ends; EF, elongation factor.

kit (Qiagen Inc., Valencia, CA). The purity and integrity of the RNA isolated were determined by UV absorbance spectrophotometry and by agarose gel electrophoresis, respectively. Restriction fragment differential display-polymerase chain reaction (RFDD-PCR) was employed to analyze gene expression in nontransformed and transformed cells. The RFDD-PCR was done using the Display Profile™ kit (Display Systems Biotech, Vista, CA) according to the manufacturer's instructions. Double-stranded cDNA synthesized from RNA was digested with *TaqI* restriction enzyme and was ligated to specifically constructed DNA adaptors of the Display Profile™ kit. The DNA templates thus prepared were subsequently PCR-amplified using Cy5-labeled 5'-primer in combination with each of the 64 display probe primers of the kit, facilitating amplification of ~25,000 distinct cDNA fragments (Display Systems Biotech). The amplified cDNA was size fractionated by 6% denaturing polyacrylamide gel electrophoresis. Following electrophoresis, gels were scanned with a Storm Fluorimager (Molecular Dynamics Inc., Sunnyvale, CA). Differentially expressed cDNA fragments were excised and eluted from the gel and PCR-reamplified using the original set of primers used in RFDD-PCR.

Northern Hybridization Analysis—PCR-reamplified cDNA was subcloned into the TA cloning vector of the TOPO™ TA cloning kit (Invitrogen, Carlsbad, CA). Differential expression of the TEF-1 δ cDNA fragment in the nontransformed and the transformed cells was confirmed by Northern hybridization analysis following standard procedures (13). The differentially expressed TEF-1 δ cDNA fragment was labeled with digoxigenin (Roche Molecular Biochemicals) by random prime labeling and was used as the probe for hybridization. The hybridized target gene was detected using the DIG-easy detection system (Roche Molecular Biochemicals) as per the procedure provided by the manufacturer.

Cloning Full-length cDNA of TEF-1 δ —The full-length cDNA of TEF-1 δ was cloned by the rapid amplification of cDNA ends (RACE) technique, using the SMART™ RACE cDNA amplification kit (CLONTECH Laboratories, Palo Alto, CA) following the protocol provided by the manufacturer. TEF-1 δ cDNA-specific primers used in the RACE cloning procedure were designed using the Gene Runner software (Hastings Software Inc., Hastings, NY) based on the DNA sequence of the differentially expressed cDNA fragment isolated from the RFDD-PCR gel. The sequences of the primers used in the RACE cloning are as follows: 5'-RACE, 5'-GCAGGTCTCACACATGAAACCACACTC-3'; 3'-RACE, 5'-GCAGCTTTCGACAAGATCTGAATGTCCGAGAG-3'. The 5' and 3'-RACE products synthesized were directly cloned into the TA cloning vector (Invitrogen, Carlsbad, CA). The DNA sequence of the RACE products was determined using T3 and T7 sequencing primers, and the data were analyzed to confirm that the sequence was same as the original cDNA fragment that was differentially expressed in the cell lines. The 5' and 3'-RACE products were digested with the restriction enzyme, *BspMI*, and the resulting fragments were ligated to obtain the full-length cDNA.

DNA Sequencing and Analysis of the Sequence Data—DNA sequencing of the TEF-1 δ cDNA at various stages of its isolation and cloning was done with an ABI PRISM automated DNA sequencer (PerkinElmer Life Sciences). DNA sequence homology searches were conducted using the BLASTN program of the National Center for Biotechnology Information (Bethesda, MD).

Transfection-mediated Overexpression of TEF-1 δ cDNA in Mammalian Cells—The open reading frame of the TEF-1 δ cDNA was fused in frame with the V5 epitope and His₆ tag of the expression vector, pcDNA3.1/V5-His-TOPO (Invitrogen, Carlsbad, CA). The nucleotide sequence, 5'-CACC-3', was added 5' to the start codon of the cDNA, and the stop codon was removed. Plasmid DNA prepared using Qiagen maxi preparation kit (Qiagen Inc., Valencia, CA) was used to transfect Chinese hamster ovary and monkey kidney COS7 cells by the calcium phosphate procedure (CLONTECH). Stable transfectants were selected by culturing the cells in medium containing G418 (200–400 μ g/ml), and cell lines were developed individually from surviving colonies. Overexpression of the cDNA-encoded protein was determined by Western blot analysis using antibody for the V5 epitope of the fusion protein. The fusion protein cross-reacting with the V5 epitope antibody was detected with an enhanced chemiluminescent kit (Amersham Biosciences, Inc.).

Transfection-mediated Transformation of NIH3T3 Cells and Analysis of Tumorigenic Potential—NIH3T3 cells exhibiting contact inhibition (ATCC, Manassas, VA) were transfected with the pcDNA3.1/V5-His-TOPO expression vector containing the entire open reading frame of TEF-1 δ cDNA by the CaPO₄ procedure (CLONTECH). The transfected cells were allowed to grow in control growth medium for 4–6 weeks for the development of transformed foci. Cell lines were developed from several independent transformed foci, and expression of

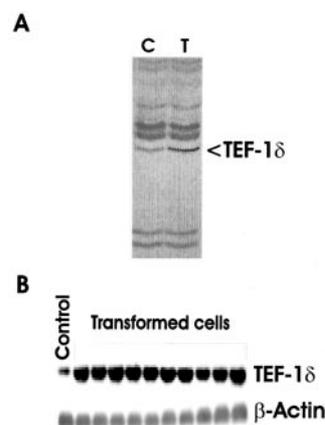


FIG. 1. Balb/c-3T3 cells transformed with CdCl₂ overexpress mouse translation elongation factor-1 δ subunit. Total RNA isolated from the control and CdCl₂-transformed Balb/c-3T3 cells were analyzed for differential gene expression by DDRT-PCR as described under "Experimental Procedures." *Panel A*, differential display analysis revealed the overexpression of a 125-bp-long cDNA fragment in the transformed cells that was identified as TEF-1 δ . *C*, control; *TC*, transformed cells. Expression of TEF-1 δ in the spontaneously transformed cells was similar to that of the nontransformed cells and is not presented; *Panel B*, Northern blot analysis of RNA isolated from control (*C*) and 10 different transformed cell lines (*TC1*–*TC10*) using TEF-1 δ cDNA as the probe confirmed overexpression of the TEF-1 δ transcript in the CdCl₂-transformed cells. Expression of TEF-1 δ in the spontaneously transformed cells was similar to that of the nontransformed cells and is not presented.

TEF-1 δ mRNA was determined by real time quantitative PCR using the ABI PRISM 7700 sequence detection system (PerkinElmer Life Sciences). The sequences of the primers used to amplify TEF-1 δ for the real time PCR analysis were as follows: 5'-AATTGGACGGGCTGGTTTG-G-3' and 5'-AGCAAGTCGGTGCCACTTTG-3'. Tumorigenic potential of the transformed cells was determined from their ability to grow as anchorage-independent colonies on soft agar (14) as well as to grow as subcutaneous tumors in athymic nude mice. For the nude mouse tumorigenesis assay, 2×10^6 cells were subcutaneously injected per mouse, and the animals were observed for a maximum period of 2 months for the development of tumors. Procedures using mice were approved by the Institutional Animal Care and Use Committee and were done according to institutional policies and regulations including the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Reversal of the Oncogenic Potential of CdCl₂-transformed Balb/c-3T3 Cells by Antisense TEF-1 δ mRNA—The TEF-1 δ cDNA was subcloned in the pcDNA3.1/V5-His-TOPO expression vector in the reverse (3'–5') orientation to construct the antisense plasmid (TEF-1 δ -AS). Subcloning of the TEF-1 δ cDNA in the reverse orientation was confirmed by restriction enzyme digestion and gel electrophoresis analysis of the digested plasmid DNA. Balb/c-3T3 cells morphologically transformed with CdCl₂ and overexpressing TEF-1 δ were transfected with the antisense plasmid DNA. Stable transfectants were selected using G418 at a concentration of 400 μ g/ml, a dose that causes 100% death of the untransfected cells. Since antibody for TEF-1 δ protein was not available, efficacy of the antisense mRNA to block the translation of TEF-1 δ mRNA could not be assessed. However, expression of the antisense TEF-1 δ mRNA in the G418-resistant cells was analyzed by RT-PCR using one primer specific for the pcDNA3.1/V5-His-TOPO vector sequence downstream to the putative transcription start and the other primer for the TEF-1 δ mRNA in the antisense orientation. The sequences of the primers used were as follows: 5'-CTGGCTTATCGAAATTAATACG-3' and 5'-TACCGGTCGATCTCTGAAC-3'. RT-PCR amplification of a transcript of 300 nucleotides is considered as the proof for the expression of TEF-1 δ antisense mRNA. Reversal of the oncogenic potential of the transformed cells expressing TEF-1 δ antisense mRNA was assessed on the basis of their ability to grow as anchorage-independent colonies on soft agar as well as subcutaneous tumors in immunodeficient nude mice as described above.

Statistical Analysis—Statistical significance of the data presented as mean \pm S.E. was analyzed by Student's *t* test or by one-way analysis of variance. The level of significance was set at $p < 0.05$.

Mouse: 1	MATNFLAHEKIWFDKFKYDDAERRFYEQMNGPVTSGSRQENGASVILRDIARARENIQKS XXX
Human: 1	MATNFLAHEKIWFDKFKYDDAERRFYEQMNGPVRGASRQENGATVILRDIARARENIQKS
Mouse: 61	LAGSSGPGASS SGPGGDHSELIVRITSLEVENQNLRGVVQDLQQAISKLEARLSSLEKSSP XX X X X X X XX
Human: 61	LAGSSGPGASS SGTSGDHGELVRIASLEVENQSLRGVVQELQQAISKLEARLNVLEKSSP
Mouse: 121	TFRATAPQTQHVS PMRQVEPPTKKGATPAEDDEDKIDILFGSDEEEEDKEAARLREERLR XX X X X X
Human: 12	GHRATAPQTQHVS PMRQVEPPAKK PATPAEDDEDKIDILFGSDNEEDKEAAQLREERLR
Mouse: 181	QYAEKAKKPTLVAKSSILLDVKPWDETDMAQLETCVRSIQLDGLVWGASKLVPVGYGI X X
Human: 181	QYAEKAKKPALVAKSSILLDVKPWDETDMAQLEACVRSIQLDGLVWGASKLVPVGYGI
Mouse: 241	RKLQIQCVVEDDKVGTDLLEEEITKFEHVQSVDIAAFDKI 281 X
Human: 241	RKLQIQCVVEDDKVGTDLLEEEITKFEHVQSVDIAAFNKI 281

FIG. 2. **Alignment of amino acid sequences of mouse and human translation elongation factor-1 δ subunits.** The deduced amino acid sequences of mouse (GenBankTM accession number AF304351) and human (GenBankTM accession number Z21507) TEF-1 δ were aligned using the BLASTP program of the National Center for Biotechnology Information (Bethesda, MD). The nonmatching amino acids are marked X. The leucine zipper region is in *boldface type* and *underlined*.

RESULTS

Overexpression of TEF-1 δ in Balb/c-3T3 Cells Morphologically Transformed with CdCl₂—Results of the differential display analysis of gene expression revealed differential expression of several genes in the cadmium-transformed cells compared with the nontransformed cells (data not shown). Consistent and reproducible overexpression of a 125-bp-long cDNA fragment in the cadmium-transformed Balb/c-3T3 cells compared with the nontransformed cells was noticed (Fig. 1A). Analysis of DNA sequence of the differentially expressed cDNA fragment showed marked similarity to human TEF-1 δ . Northern hybridization of the differentially expressed cDNA fragment to RNA isolated from control and transformed cells detected a transcript of ~1.0 kb that was found overexpressed in the transformed cells. Furthermore, overexpression of the transcript was detected in 100% of the transformed cell lines (10 of 10) that were developed from individual transformed foci (Fig. 1B). Similar overexpression of the transcript was also observed in the cell lines derived from tumors developed in nude mice injected subcutaneously with the cadmium-transformed Balb/c-3T3 cells (data not presented).

Cloning and Transfection-mediated Overexpression of TEF-1 δ cDNA—Comparison of the nucleotide sequence of the cDNA fragment by BLASTN analysis of NCBI identified the differentially expressed cDNA as TEF-1 δ . Cloning the full-length cDNA by SMART-RACE technique resulted in isolation of a 5'-RACE product consisting of 956 nucleotides and a 3'-RACE product consisting of 109 nucleotides. Subsequent restriction enzyme digestion of the 5'- and 3'-RACE products and ligation of the digested DNA fragments resulted in cloning the full-length cDNA consisting of 1004 nucleotides. Analysis of the DNA sequence revealed a reading frame encoding for the predicted protein consisting of 281 amino acids (the nucleotide and amino acid sequences that are not presented here are available in the GenBankTM, accession number AF304351). Analysis of the deduced amino acid sequence revealed the presence of a leucine zipper (Fig. 2). Transfection of monkey kidney COS7 cells and Chinese hamster ovary cells with pcDNA3.1 expression plasmid containing the entire reading frame for the TEF-1 δ cDNA resulted in the expression of a protein with an approximate molecular mass of 31 kDa (Fig. 3).

Transfection-mediated Overexpression of TEF-1 δ and Morphological Transformation of NIH3T3 Cells—Transfection of NIH3T3 cells with the pcDNA3.1 expression plasmid contain-

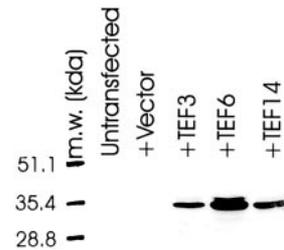


FIG. 3. **Transfection and expression of mouse TEF-1 δ cDNA in mammalian cells.** Chinese hamster ovary cells were transfected either with the vector pcDNA3.1/D/V5-His-TOPO alone (+Vector) or with the vector containing the entire open reading frame of TEF-1 δ cDNA (+TEF) as described under "Experimental Procedures." 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 ~34.2 kDa, of which 3.6 kDa corresponds to the V5 epitope and the polyhistidine region derived from the vector. TEF3, TEF6, and TEF14 are three different cell lines permanently expressing the TEF-1 δ -V5 epitope fusion protein. Similar results were obtained with monkey kidney COS7 cells transfected with the TEF-1 δ plasmid DNA (data not shown).

ing the TEF-1 δ cDNA resulted in overexpression of TEF-1 δ mRNA (Fig. 4A) and the 31-kDa protein encoded by the cDNA (results not shown). Overexpression of the cDNA-encoded protein, furthermore, resulted in morphological transformation of NIH3T3 cells as evidenced by the appearance of transformed foci (Fig. 4, B and C). The oncogenic potential of the transformed foci was confirmed by anchorage-independent growth of cell lines derived from the foci (Fig. 5A). Similarly, these cells also exhibited tumorigenic potential as evidenced by their development to subcutaneous tumors in nude mice (Fig. 5, B and C).

Expression of Antisense TEF-1 δ mRNA and the Oncogenic Potential of CdCl₂-transformed Balb/c-3T3 Cells—Culturing Balb/c-3T3 cells morphologically transformed with CdCl₂ in medium containing G418 (400 μ g/ml) resulted in 100% cell death. Transfection of the cells with the pcDNA 3.1 vector either alone or containing the TEF-1 δ cDNA in the antisense orientation provided protection to the cells from the cytotoxicity of G418 as evidenced by the absence of cell death in the transfected cells. Thus, the ability to grow in medium containing G418 was considered as an indication for the presence and the subsequent expression of the plasmid DNA in the transfected cells. Results of RT-PCR analysis detected the antisense

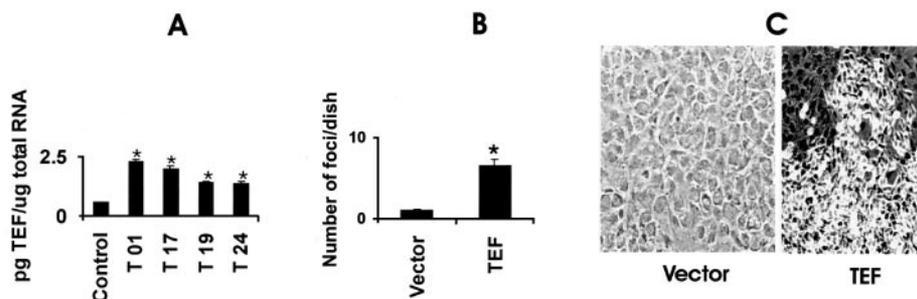


FIG. 4. **Transfection-mediated overexpression of mouse TEF-1 δ is oncogenic to NIH3T3 cells.** NIH3T3 cells exhibiting contact inhibition were transfected with the vector, pcDNA 3.1D/V5-His-TOPO alone (Vector) or the vector containing the TEF-1 δ cDNA (TEF) as described under "Experimental Procedures." Transfection-mediated overexpression of TEF-1 δ protein resulted in transformation of NIH3T3 cells as evidenced by the appearance of transformed foci. *A*, expression of TEF-1 δ mRNA in the NIH3T3 cells as analyzed by real time PCR demonstrated significant overexpression of TEF-1 δ in the transformed cells (T01, T17, T19, and T24 are cell lines developed from four independent transformed foci) compared with the nontransformed cells (results of the Western blot analysis detecting the 34.2-kDa fusion protein are similar to those presented in Fig. 3 and are not presented). *B*, number of transformed foci in cells transfected either with the vector or with the TEF-1 δ plasmid DNA; *C*, photomicrograph of transfected cells showing transformed foci. The foci were fixed with methanol and stained with methylene blue and were observed under a microscope. *, statistically significant compared with the control ($p < 0.05$), where $n = 4$.

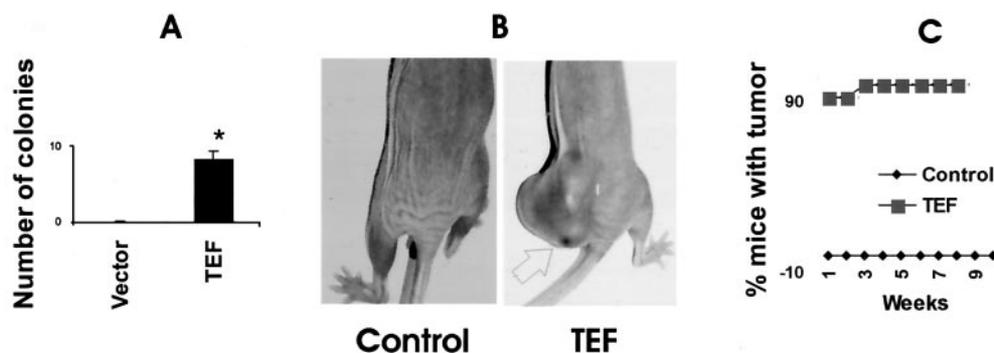


FIG. 5. **Oncogenic potential of NIH3T3 cells overexpressing mouse translation elongation factor-1 δ .** NIH3T3 cells were transfected either with the vector pcDNA 3.1D/V5-His-TOPO alone (Vector) or with the vector containing the TEF-1 δ cDNA (TEF) as described under "Experimental Procedures." The transfected cells were tested for their capacity to grow as anchorage-independent colonies on soft agar (*A*) as well as for their potential to grow as subcutaneous tumors in nude mice (*B* and *C*). Similar results were obtained when experiments were conducted using four different cell lines, and results presented are representative of one of the cell lines used. *, statistically significant compared with the control ($p < 0.05$), where $n = 4$.

transcript of TEF-1 δ in the cells transfected with the antisense plasmid DNA (Fig. 6A). Expression of the antisense RNA against TEF-1 δ mRNA in the CdCl₂-transformed Balb/c-3T3 cells overexpressing TEF-1 δ resulted in a significant reversal of the oncogenic potential of the cells. This was evidenced by a 40% decrease in the number of anchorage-independent colonies growing on soft agar (Fig. 6B) as well as by the significant decline in the tumorigenic potential in nude mice injected with the cells (Fig. 6C). In addition to a significant delay in the onset and visual appearance of tumors, a significant reduction in size of the tumors was also observed in the mice injected with the TEF-1 δ antisense mRNA-expressing cells compared with the corresponding controls (Fig. 6D).

DISCUSSION

Alterations in the expression of genes, especially those regulating the cell cycle, are critical in the development of malignant transformation (15, 16). Modifications in the translational machinery of cells, including changes in both eukaryotic translation initiation factors and elongation factors, can also result in susceptibility to transformation and the acquisition of transformed and oncogenic properties by cells (17, 18). Translation elongation factors constitute a group of nucleotide exchange proteins that bind GTP and aminoacyl-tRNA and result in codon-dependent placement of the aminoacyl-tRNA on the ribosome resulting in peptide chain elongation (17). Enhanced expression of elongation factor-1 α (EF-1 α) confers susceptibility to carcinogen and UV light-induced transformation in mouse and Syrian hamster cell lines (19). Furthermore, ele-

vated levels of EF-1 α and EF-1 γ are found in tumors of the pancreas, colon, breast, lungs, prostate, and stomach relative to normal tissues (20–24). Results of this study that agree with these reports indicate that alteration in expression of the translation elongation factors, including mouse TEF-1 δ , contribute to cell transformation and cancer development.

TEF-1 δ cDNA has been cloned previously from several species (25, 26). We have cloned and characterized the mouse TEF-1 δ cDNA, and comparison of the nucleotide and predicted amino acid sequence revealed that the mouse TEF-1 δ cDNA exhibits the highest similarity to human TEF-1 δ cDNA, 92 and 76% similarity to the amino acid and to the nucleotide sequence, respectively. The open reading frames of both mouse and human TEF-1 δ cDNAs possess 843 nucleotides encoding for the predicted peptide consisting of 281 amino acids with a calculated molecular weight of 31 kDa. Similar to human TEF-1 δ , mouse TEF-1 δ also possesses an EF-1 β conserved domain, an EF-1 guanine nucleotide exchange domain, and a leucine zipper motif. Similarity between the mouse TEF-1 δ and human TEF-1 δ proteins is more conserved in the C terminus, especially in the EF-1 β domain and in the guanine nucleotide exchange domain. With the exception of the leucine zipper region, the N-terminal sequence is less conserved between human and mouse TEF-1 δ . A similar observation has been reported for TEF-1 δ protein of human, *Xenopus*, and *Artemia* (25).

Results of the various experiments conducted in the present study have clearly demonstrated that TEF-1 δ is a cellular

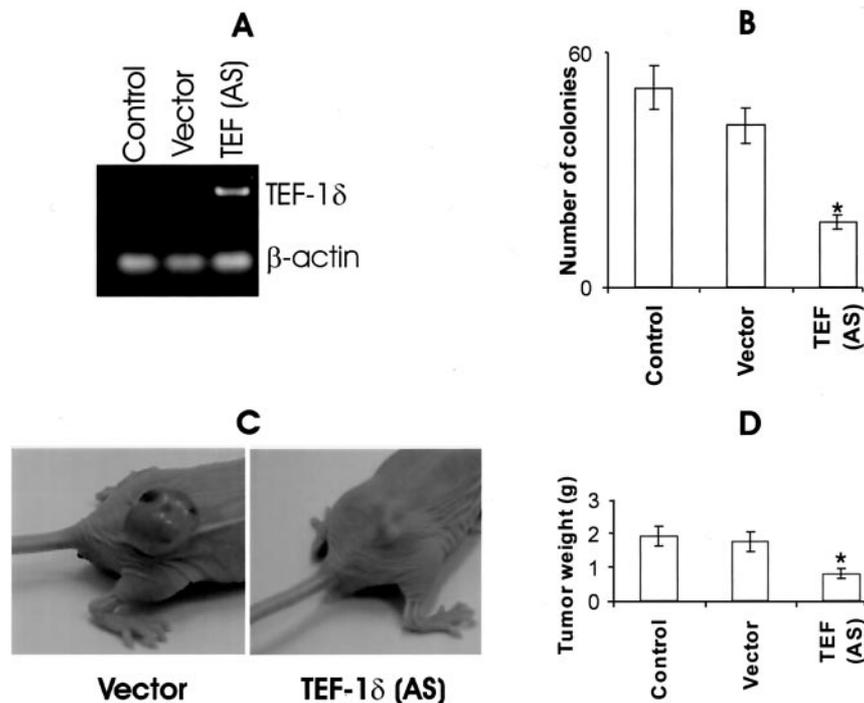


FIG. 6. Reversal of the oncogenic potential of CdCl₂-transformed Balb/c-3T3 cells by TEF-1δ antisense RNA. Balb/c-3T3 cells, transformed with CdCl₂, were transfected with the pcDNA3.1 vector alone (Vector) or with the vector expressing the antisense TEF-1δ mRNA (TEF (AS)). Stable transfectants were selected using G418 (400 μg/ml). **A**, stable transfectants were analyzed by RT-PCR for the expression of TEF-1δ antisense mRNA using primers as described under "Experimental Procedures." β-Actin was used as the housekeeping gene; **B**, 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), and colonies larger than 0.1 mm were quantitated. **C**, 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 (antisense) plasmid are shown. **D**, at the end of 4 weeks after the subcutaneous injection, the tumors developed were surgically removed. The tumors were weighed, and the results are presented. Similar results were obtained when experiments were conducted using three different cell lines, and results presented are representative of one of the cell lines used. *, statistically significant ($p < 0.05$), where $n = 4$.

proto-oncogene. Significant overexpression of TEF-1δ was observed in all (10 of 10) CdCl₂-transformed Balb/c-3T3 cell lines analyzed. Similar overexpression of TEF-1δ was also observed when these cells were injected into nude mice and cell lines were developed from the tumors (data not presented). Further evidence for the oncogenic potential of TEF-1δ is obtained from experiments involving NIH3T3 cell transformation. Transformation of NIH3T3 cells in conjunction with nude mouse tumorigenesis assay is a very well established procedure to confirm the oncogenic potential of genes (27). Transfection of NIH3T3 cells with the expression plasmid containing TEF-1δ cDNA resulted in overexpression of the encoded protein and this was associated with cell transformation as evidenced by the appearance of transformed foci. The oncogenic potential of TEF-1δ-overexpressing NIH3T3 cells was further confirmed by their potential for anchorage-independent growth on soft agar and for tumorigenesis in nude mice. Furthermore, blocking the translation of TEF-1δ mRNA with its antisense mRNA resulted in significant reversal of the oncogenic potential of the CdCl₂-transformed Balb/c-3T3 cells. These results suggest that the overexpression of TEF-1δ was in fact the causative factor for CdCl₂-induced cell transformation and tumorigenesis rather than simply being a consequence of the metal-induced malignant transformation.

Despite the data presented in this paper documenting the involvement of elevated expression of TEF-1δ in the cadmium-induced cell transformation and tumorigenesis, neither the mechanisms of its overexpression nor those responsible for the resulting malignant transformation are known. In general, cadmium-induced deregulation of gene expression is mediated through the generation of reactive oxygen species (11), alter-

ations in cellular calcium levels (11), modulation of the expression of cellular kinases (28, 29), and the generation of mitogenic stimuli (30). Whether any of the aforementioned mechanisms are actually involved in the cadmium-induced up-regulation of transcription of TEF-1δ is open for investigation.

Results of previous studies have shown that qualitative and quantitative changes in expression of translation elongation factors, in general, are responsible for their role in carcinogenesis. For example, in *Saccharomyces cerevisiae*, mutations of EF-1α lead to loss of proofreading potential, resulting in translational infidelity (31, 32) that is known to result in carcinogenesis (33). The reported involvement of elongation factors in senescence and mitosis may also be relevant in understanding their proposed role in chemical carcinogenesis. In *Drosophila melanogaster*, for example, the cellular level and catalytic activity of EF-1α are lower during senescence (34), and forced expression of EF-1α results in extension of life span compared with control flies (35). Thus, it appears that overexpression of elongation factors can result in a proliferative response that is characteristic of cell transformation during chemical carcinogenesis. This is further supported by the role of elongation factors in cell division through their association with the mitotic spindle apparatus (36). Thus, in summary, our results demonstrate that TEF-1δ is a novel cellular proto-oncogene, although the actual cellular/molecular mechanisms responsible for its oncogenic function need to be clarified.

The results of our study may have potential clinical value. It may be worth exploring the diagnostic and therapeutic implications of our results demonstrating the role of TEF-1δ overexpression in cell transformation and tumorigenesis induced by CdCl₂. This will involve studies designed to clarify whether the

overexpression of TEF-1 δ is unique to cadmium-induced carcinogenesis or is a general feature of any malignant transformation. A positive correlation between the overexpression of TEF-1 δ and the incidence of tumorigenesis may facilitate the use of TEF-1 δ overexpression as a diagnostic indicator or as a biological marker for carcinogenesis. The observation that expression of the antisense TEF-1 δ mRNA could reverse the tumorigenic potential of the CdCl₂-transformed Balb/c-3T3 cells is of potential therapeutic value. Intervention of TEF-1 δ overexpression may represent an effective approach for the therapy of tumors overexpressing TEF-1 δ .

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Pius Joseph, Yi-Xiong Lei, Wen-Zong Whong and Tong-Man Ong

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