Abnormal Expression of Eukaryotic Translation Factors in Malignant Transformed Human Bronchial Epithelial Cells Induced by Crystalline Nickel Sulfide

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Objective To study the oncogenic potential of mouse translation initiation factor 3 (TIF3) and elongation factor-1δ (TEF-1δ) in malignant transformed human bronchial epithelial cells induced by crystalline nickel sulfide (NiS).

Methods Abnormal expressions of human TIF3 and TEF-1δ genes in two kinds of NiS-transformed cells and NiS-tumorigenic cell lines were investigated and analyzed by the reverse transcript polymerase chain reaction (RT-PCR) and fluorescent quantitative polymerase chain reaction (FQ-PCR), respectively.

Results RT-PCR analysis primarily showed that both human TIF3 and TEF-1δ mRNA expressions in two kinds of NiS-transformed cells and NiS-tumorigenic cell lines were increased as compared with controls. FQ-PCR assay showed that the levels of TIF3 expressions in the transformed cells and tumorigenic cells were 3 and 4 times higher respectively, and the elevated expressions of TEF-1δ cDNA copies were 2.7- to 3.5-fold in transformed cells and 4.1- to 5.2-fold in tumorigenic cells when compared with non-transformed cells, indicating that the over-expressions of human TIF3 and TEF-1δ genes were related to malignant degree of the cells induced by nickel.

Conclusions These findings demonstrate that there are markedly abnormal expressions of TIF3 and TEF-1δ genes during malignant transformation of human bronchial epithelial cell lines induced by crystalline NiS. They seem to be the molecular mechanisms potentially responsible for human carcinogenesis due to nickel.

Key words: Nickel compounds; Human cells; Transformation; Translation factors; Fluorescent quantitative PCR

INTRODUCTION

Metallic nickel and nickel compounds are known environmental and occupational metals. The available epidemiological data have shown that nickel alloy and compounds can cause allergic diseases, lung and nasal cancer in human beings, especially in those working in nickel industries such as nickel refineries, stainless steel welding, calcining and sintering operations[1-3]. Experimental studies have demonstrated that the carcinogenicity of nickel compounds may be in part due to their ability to cause DNA damages such as DNA strand breaks, DNA-DNA crosslinks, DNA-protein crosslinks, infidelity of DNA replication and inhibition of DNA repair as well as abnormal DNA synthesis[4-5]. The International Agency for Research on Cancer (IARC) has classified nickel compounds as a category I carcinogen and metallic nickel is considered possible carcinogenic to human beings[6-7]. Even though nickel compounds are known carcinogens, the underlying carcinogenic mechanisms, however, are still not fully understood[8].

Recent developments in oncogenomics have illustrated the usefulness of carcinogen-induced changes in gene expression to understand the potential cellular and molecular mechanisms of chemical carcinogenesis. Recently, Joseph et al.[9-10] and Lei et al.[11-12] have identified, cloned, and characterized the mouse TIF3 (GenBank Accession Number AF271072) and TEF-1δ (GenBank Accession Number AF304351) as two novel cadmium-responsive proto-oncogenes. The objective of the present research was to determine if the abnormal expressions of TIF3 and TEF-1δ genes were present during malignant transformation of...
human bronchial epithelial cell lines (16HBE) induced by crystalline nickel sulfide (NiS) with qualitative and quantitative methods, and to further explore the potential carcinogenesis of insoluble nickel compounds and the probability of human TIF3 and TEF-1δ as molecular biomarkers of metal expression situation for human population.

MATERIALS AND METHODS

Preparation of Nickel-transformed Human Cell Lines

Malignant transformation experiments of human bronchial epithelial cells (16HBE) induced by insoluble crystalline nickel sulfide (NiS) were performed as previously described\[^{13}\]. Briefly, the cells seeded in dishes were treated with different concentrations of nickel compounds (0.25-2.00 μg/cm\(^2\)). Minimum essential medium (MEM) was used as a solvent control and benzo[\(a\)]pyrene (B[a]P) (0.2 μg/mL) was used as a positive control. The concentrations of nickel compounds, which gave the relative colony forming efficiencies (RCFEs) from 5%-90%, were selected for the morphological transformation studies. Malignant cell transformation assay was performed according to Dunkel’s recommended procedure\[^{14}\]. The transformed foci induced by insoluble crystalline NiS at the concentration of moderate cytotoxicity, were individually isolated for soft agar assay and nude mouse tumorigenicity experiments. The established NiS-transformed cell lines and NiS-tumorigenic cell lines were used to investigate the abnormal expressions of TIF3 and TEF-1δ genes, respectively.

Total RNA Isolation and Primer (probe) Sequences

The medium from the flasks containing the monolayer of NiS-transformed cell lines, NiS-tumorigenic cell lines and non-transformed cells was completely aspirated and rinsed with phosphate buffered saline (1×PBS) 1-2 times. Total RNA from the cells was isolated using the TRIzol kit (Gibco BRL, USA) in accordance with the instructions provided by the manufacturer. The purity and integrity of the total RNA, used for reverse transcription of mRNA to cDNA, were analyzed by spectrophotometry (Eppendorf, Germany) and agarose gel electrophoresis, respectively. For detecting expressions of human TIF3 and TEF-1δ cDNA with qualitative and quantitative assays, the primers of human TIF3 (GenBank Accession Number Z21507) and TEF-1δ (GenBank Accession Number U39067), and the corresponding probes are listed in Tables 1 and 2.

**TABLE 1**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers and β-actin Sequences(^a)</th>
<th>Length of Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIF3</td>
<td>Sense: 5’-gTCACAgCCggATgAACCC-3’</td>
<td>770 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-ATggCTTCACTgACCACCgCC-3’</td>
<td></td>
</tr>
<tr>
<td>TEF-1δ</td>
<td>Sense: 5’-TCCCATgCgCCAAgTggAgC-3’</td>
<td>103 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-CTCCTCCCATTgTCACTgC-3’</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Sense: 5’-AggCATTgATgAACTCCg-3’</td>
<td>301 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-AgTgATgACCTggCCgTCAg-3’</td>
<td></td>
</tr>
</tbody>
</table>

*Note. RT-PCR: Reverse-Transcript-Polymerase Chain Reaction Technique. \(^a\)designed with GENE-RUNNER software and synthesized by Invitrogen Co. USA.

**TABLE 2**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers and Probe Sequences(^a)</th>
<th>Length of Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIF3</td>
<td>Sense: 5’-gCCTCTCCTCCTAaCTATgA-3’</td>
<td>65 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-gTggTTACATCCATggCTTCCCT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’-FAM-CATgTggTTCCACTgGgTgT-TAMARA-3’</td>
<td></td>
</tr>
<tr>
<td>TEF-1δ</td>
<td>Sense: 5’-gCTACAAACTTCCATgCACAATgAgAgG-3’</td>
<td>86 bp</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-TTCATCTgTgCTgATgAACATCTCCTTT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 5’-FAM-TggTTACAAAgTTCAATATgAGG- TAMARA-3’</td>
<td></td>
</tr>
</tbody>
</table>

*Note. FQ-PCR: Fluorescent Quantitative-Polymerase Chain Reaction Assay. \(^a\)designed with Primer Express 2.0 software and synthesized by Da’an Co. China.
Observation of TIF3 and TEF-1δ by RT-PCR Technique

The total RNA of TIF3 and TEF-1δ was diluted to the concentration of 1 mg/mL for reverse transcript (RT) experiments. According to GenBank, the forward and reverse primers of both TIF3 and TEF-1δ cDNA were designed with GENE-RUNNER software and synthesized by Invitrogen Co. USA for the polymerase chain reaction (PCR). The primer sequences of β-actin gene were designed as the internal standard (Table 1). Using the Thermoscript™ RT kit (Gibco BRL, USA) and PCR amplification kit (TaKaRa, Japan) according to their manufacturer’s instructions, the abnormal expressions of TIF3 and TEF-1δ mRNA were primarily investigated by RT-PCR technique. The RT-PCR products of TIF3 and TEF-1δ in non-transformed cells, NiS-transformed cells and NiS-tumorigenic cells under the internal standard of β-actin were detected by agarose gel electrophoresis, and scanned with a densitometer (Molecular Dynamics, Sunnyvale, CA, USA) for semi-quantification.

Detection of TIF3 and TEF-1δ by FQ-PCR Assay

Preparation of positive templates standard TIF3 or TEF-1δ cDNA generated by reverse transcription from target RNA was amplified as positive template standard in PE9600 PCR machine. 5×PCR buffer [10 mmol/L Tris-HCl (pH 8.0), 50 mmol/L KCl, 2 mmol/L MgCl2], was mixed with 20 μmol/L Taq DNA polymerase, 10 mmol/L dNTPs and 25 μmol/L primers, and then 5 μL of TIF3 or TEF-1δ cDNA template (corresponding to 1.25 μg RNA) was added to the master mix. PCR amplification was performed with pre-denaturation (93°C for 2 min), and then 40 cycles of denaturation (93°C for 45 s), annealing (55°C for 45 s) and extension (72°C for 1 min). The copies (copies/μg RNA) were automatically calculated (corresponding to 1.25 μg RNA). Then, 50 μL of total reaction system was performed with pre-denaturation (93°C for 2 min), and then 40 cycles of denaturation (93°C for 45 s), annealing (55°C for 45 s). Every time when FQ-PCR of the examined samples were performed, TIF3 or TEF-1δ cDNA standard gradient templates of 10^0, 10^1, 10^2, 10^3, 10^4 copies/μL were simultaneously amplified and the blank PCR reagents were simultaneously amplified as negative controls. Three replicates of each sample were processed. Fluorescence was measured at the end of the annealing-extension phase of each cycle. The fluorescent intensity at 518 nm wavelength was detected automatically by the PE 7000 sequence detection system, and the amounts of target cDNA (copies/μg RNA) were automatically calculated according to standard gradient template curve and the amount of target cDNA by comparing a threshold value. The threshold value (background) for the fluorescence of all samples was set manually. The reaction cycle at which the PCR product exceeded this fluorescence threshold was identified as the threshold cycle (CT).

Calculation of TIF3 and TEF-1δ cDNA

The amounts of target cDNA (copies/μg RNA) were calculated with the formula according to standard gradient template curve and the amount of target cDNA. C=Q × V_cDNA/(V RNA × L RNA), where C represents TIF3 or TEF-1δ cDNA copies, Q represents amounts of target cDNA examined, V_cDNA represents volume of cDNA template, V RNA represents volume of RNA template for reverse transcript, L RNA represents concentration of RNA template.

Statistical Analysis of Data

Differences among groups were statistically analyzed by one-way analysis of variance (ANOVA) or Dunnett test using Statistical Package for the Social Sciences (SPSS 11.0). Data were presented as x ± s. P<0.05 was considered statistically significant.

RESULTS

Preliminary Analyses of TIF3 and TEF-1δ Expressions

The results of RT-PCR experiments primarily showed that the expressions of TIF3 and TEF-1δ cDNA in NiS-transformed cells and NiS-tumorigenic cells were slightly higher than those in the non-transformed control cells. After densitometric quantification and normalization with β-actin, the relative
mass of TIF3 bands was 0.52, 0.80, and 0.83 in non-transformed cells, transformed cells and tumorigenic cells, respectively. Under the same condition, the relative mass of TEF-1δ bands was 0.25, 0.31, and 0.37 respectively in non-transformed cells, transformed cells and tumorigenic cells. These semi-quantitative analyses primarily indicated that abnormal expressions of TIF3 and TEF-1δ occurred in transformed cells and tumorigenic cells induced by crystalline nickel sulfide (NiS) (Figs. 2 and 3).

Quantitative Analysis of TIF3 Gene Expression

The overexpressions of TIF3 genes in human bronchial epithelial cells induced by crystalline NiS were further quantified by FQ-PCR assay. The levels of TIF3 expressions in the Nis-transformed cells and NiS-tumorigenic cells were 3 and 4 times higher respectively than those in control cells (P<0.05 or P<0.01), indicating that expression level of TIF3 gene was related to malignant degree of the cells (Table 3 and Fig. 4).

Quantitative Analysis of TEF-1δ Gene Expression

As shown in Table 4 and Fig. 5, FQ-PCR assay further revealed that the levels of TEF-1δ cDNA copies were from 2.7- to 3.5-fold in transformed cells and from 4.1- to 5.2-fold in tumorigenic cells when compared with non-transformed cells (P<0.05 or P<0.01), indicating a relation of overexpression of TEF-1 δ gene to malignant degree of the cells.
induced by nickel.

**TABLE 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>TIF3 cDNA (× 10^6 copies/μg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transformed Cells</td>
<td>3</td>
<td>Cell Line 1 (% of Control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.98 ± 0.60 (100%)</td>
</tr>
<tr>
<td>NiS-transformed Cells</td>
<td>3</td>
<td>Cell Line 2 (% of Control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.44 ± 0.48 (100%)</td>
</tr>
<tr>
<td>NiS-tumorigenic Cells</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.46 ± 0.87 (284%)^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.41 ± 0.66 (304%)^b</td>
</tr>
</tbody>
</table>

Note. Compared with control cells, ^aP < 0.05, ^bP < 0.01 (Analysis of variance and Dunnett-test).

**TABLE 4**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>TEF-1 δ cDNA (× 10^6 copies/μg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transformed Cells</td>
<td>3</td>
<td>Cell Line 1 (% of Control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.43 ± 0.27 (100%)</td>
</tr>
<tr>
<td>NiS-transformed Cells</td>
<td>3</td>
<td>Cell Line 2 (% of Control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.30 ± 0.33 (100%)</td>
</tr>
<tr>
<td>NiS-tumorigenic Cells</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.05 ± 0.76 (351%)^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.49 ± 3.20 (267%)^c</td>
</tr>
</tbody>
</table>

Note. Compared with control cells, ^bP<0.05, ^cP<0.01 (Analysis of variance and Dunnett-test).
FIG. 4. Fluorescent quantitative PCR assays of human *TIF3* gene from non-transformed cells, NiS-transformed cells and NiS-tumorigenic cells. The X axis denotes the cycle number of a quantitative PCR reaction, and the Y axis denotes the ΔRn, which is the fluorescence intensity after normalization with the threshold (background).

FIG. 5. Fluorescent quantitative PCR assays of human *TEF-1δ* gene from non-transformed cells, NiS-transformed cells and NiS-tumorigenic cells. The X axis denotes the cycle number of a quantitative PCR reaction, and the Y axis denotes the ΔRn, which is the fluorescence intensity after normalization with the threshold (background).

**DISCUSSION**

Alterations in the expression of genes, especially the genetic changes in cell cycle, are critical in the development of malignant transformation[17-18]. 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 in cells[19-20]. For example, the largest eukaryotic translation initiation factor, eIF3, consists of at least ten subunits ranging in mass from 35 to 170 kDa. eIF3 can bind to the 40 S ribosome in an early step of translation initiation and promote the binding of methionyl-tRNA and mRNA[21]. Enhanced expressions of eIF3 subunits are found among transformed, tumorous and tumor tissues[22-23]. Similarly, translation elongation factors constitute a group of nucleotide exchange proteins that bind to GTP and aminoacyl-tRNA and lead to codon-dependent placement of the aminoacyl-tRNA on the ribosome resulting in peptide chain elongation[19]. Enhanced expression of elongation factor-1α (EF-1α) confers susceptibility to carcinogen and UV light-induced transformation to mouse and Syrian hamster cell lines[25]. Furthermore, elevated levels of EF-1α and EF-1γ are found in
tumors of the pancreas, colon, breast, lungs, prostate and stomach relative to normal tissues. 

Recently, the mouse TIF3 and TEF-1δ have been identified as two novel cadmium-responsive proto-oncogenes. Results presented in this study, compared with non-transformed human bronchial epithelial cells, have shown that the transformed cells and tumorigenic cells can express high levels of human TIF3 and TEF-1δ genes. Furthermore, overexpressions of the two genes in tumorigenic cells are higher than those in transformed cells as compared with control cells. These findings demonstrate for the first time that there are markedly abnormal expression of TIF3 and TEF-1δ genes during malignant transformation of human bronchial epithelial cell line induced by crystalline NiS, and the human TIF3 and TEF-1δ expressions are associated with malignant degree of the cells. These may be one of the molecular mechanisms potentially responsible for carcinogenesis due to nickel.

Although our previous and present studies have demonstrated the oncogenic potential of TIF3 and TEF-1δ, the cellular mechanisms underlying overexpression of TIF3 and TEF-1δ as well as cell transformation and tumorigenesis are not known. It may be worth exploring the expressions of these two genes during malignant transformation of human bronchial epithelial cell lines induced by crystalline NiS as well as changes in risk population. But it seems that abnormal expression of human TIF3 and TEF-1δ can be used as a molecular biomarker of metal expression situation in human beings.

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