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Alterations of FHIT Gene and P16 Gene in Nickel Transformed Human Bronchial Epithelial Cells[1]

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Objective To study the alterations of FHIT gene and P16 gene in malignant transformed human bronchial epithelial cells induced by crystalline nickel sulfide using an immortal human bronchial epithelial cell line, and to explore the molecular mechanism of nickel carcinogenesis. **Methods** 16HBE cells were treated 6 times with different concentrations of NiS *in vitro*, and the degree of malignant transformation was determined by assaying the anchorage-independent growth and tumorigenicity. Malignant transformed cells and tumorigenic cells were examined for alterations of FHIT gene and P16 gene using RT-PCR, DNA sequencing, silver staining PCR-SSCP and Western blotting. **Results** NiS-treated cells exhibited overlapping growth. Compared with that of negative control cells, soft agar colony formation efficiency of NiS-treated cells showed significant increases ($P<0.01$) and dose-dependent effects. NiS-treated cells could form tumors in nude mice, and a squamous cell carcinoma was confirmed by histopathological examination. No mutation of exon 2 and exons 2-3, no abnormal expression in p16 gene and mutation of FHIT exons 5-8 and exons 1-4 or exons 5-9 were observed in transformed cells and tumorigenic cells. However, aberrant transcripts or loss of expression of the FHIT gene and Fhit protein was observed in transformed cells and tumorigenic cells. One of the aberrant transcripts in the FHIT gene was confirmed to have a deletion of exon 6, exon 7, exon 8, and an insertion of a 36 bp sequence replacing exon 6-8. **Conclusions** The FHIT gene rather than the P16 gene, plays a definite role in nickel carcinogenesis. Alterations of the FHIT gene induced by crystalline NiS may be a molecular event associated with carcinogen, chromosome fragile site instability and cell malignant transformation. FHIT may be an important target gene activated by nickel and other exotic carcinogens.

Key words: Crystalline nickel sulfide; Human bronchial epithelial cell line; Malignant transformation; P16 gene; FHIT gene

INTRODUCTION

Nickel is a widely distributed occupational and environmental pollutant. Epidemiological studies demonstrated that the incidence of respiratory tract cancer is correlated with worksite exposure to nickel. Nickel is also a potent carcinogen in laboratory animals. The International Agency for Research on Cancer (IARC) has classified nickel compounds as confirmed human carcinogens[1]. However, the mechanism of nickel carcinogenesis remains unknown. Insoluble nickel compounds such as crystalline nickel sulfide and nickel oxide have significantly higher carcinogenic potential than soluble nickel compounds[2-3]. The effect is probably associated with the amount of Ni delivered into cells, when Ni-containing particles are phagocytosed and dissolved inside cells[4-5]. While the carcinogenicity of nickel compounds has been extensively studied in experimental animals and in a variety of cultured mammalian cells[6], there are only sporadic reports of nickel carcinogenesis in cultured human cells[7-8], especially in human bronchial epithelial cells.

Since Ni has a low mutagenic potential in many mutational assays[9], and may exert carcinogenic activity by modulating gene expression, involving activation of oncogenes and inactivation of tumor suppressor genes[10]. Alterations of a number of oncogenes (ras, myc, AP-1, *etc*.) and tumor suppressor genes (P53, Rb, senescence gene) have been induced by nickel, but the relationship between the tumor suppressor genes P16 and FHIT and nickel carcinogenesis are seldom reported. In the present study, we studied the alterations of the FHIT and P16 genes in the malignant transformation of human bronchial epithelial cells induced by crystalline nickel sulfide using an immortal human bronchial epithelial

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cell line.

MATERIALS AND METHODS

Chemicals

Crystalline NiS was purchased from Johnson Matthey Catalong Ltd. The NiS particles with their diameters $\leqslant 2.5$ μm, as estimated by scanning microscopy, were obtained by grinding the compound in an agate mortar. Anti-benzo (a) pyrene-trans-7.8-dihydrodiol-9, 10-epoxide (anti-BPDE) was obtained from National Cancer Institute Chemical Carcinogen Reference Standard Repository (Kansas City, MO, USA).

Cell Culture and Transformation Protocols

The human bronchial epithelial cell line (16HBE) immortalized by SV40 large T antigen was kindly provided by Dr. XU from the Guangzhou Institute of Respiratory Diseases. Cells were grown in 25 cm^2 flasks in minimum essential medium (MEM) supplemented with 10% fetal calf serum and maintained at 37℃ in humidified air with 5% CO_2. The cultures were transferred every 3-4 days at 1:4 dilution after the monolayers reached 85% confluence.

For cytotoxicity studies with crystalline NiS, 200 cells were plated in each 60 mm plate (5 plates at each NiS concentration). After 24 h, the media were removed and replaced with media containing the appropriate concentration (0-2 μg/cm^2) of crystalline NiS. Following 48 h exposure to the compound, the plates were washed 3 times with 3 mL PBS and replaced with 3 mL of MEM with 10% fetal calf serum (FCS). After 10 days the plates were washed with PBS and then stained with 1% crystal violet. The number of colonies containing at least 50 cells was counted, and relative colony formation efficiency (RCE) was calculated. Concentrations of crystalline NiS leading to 10%-90% RCE were chosen for cell transformation.

For transformation, 2.5×10^5 16HBE cells were plated in 25 cm^2 flasks, maintained for 24 h and then treated with different concentrations of crystalline NiS in MEM with 10% FCS for 24 h and 48 h. The medium was removed. The cells were washed 2 times with PBS and grown in fresh MEM with 10% FCS. After the monolayers reached 85% confluence, the cultures were split 1:4. The cells were treated 6 times with NiS for over 20 days. Anti-BPDE-treated cells served as positive control.

Assay for Anchorage Independent Growth

In the assay for anchorage independence growth at the 35th passage, 1×10^3 cells were plated in 3 mL 0.3% agar in MEM with 20% calf bovine serum overlaid onto a solid layer of 0.6% agar in MEM with 20% calf bovine serum. The cultures were maintained for 3 weeks and colonies were counted. The plating efficiency in agar was determined by dividing the number of colonies obtained from per plate by the number of cells plated. Two anchorage-independent colonies were picked, pooled and expanded in culture and maintained as a cell line designated as "SA".

Tumorigenicity Assay

The transformed cell line ("SA" cell line) and negative control cell line were suspended in serum free medium at a density of 4×10^7 cell/mL, and 0.2 mL was injected into the flanks of 4-5 week-old athymic nude mice (4 for each experiment). Tumor growth was monitored on a weekly basis. After 3 weeks, tumorigenicity of cells was observed, and tumor tissues were examined by histopathology. Major portions of the primary tumors were dissected aseptically from the primary sites of nude mice, freed of connective tissue and rinsed with PBS containing penicillin and streptomycin. The sections of each tumor were placed in a 60 mm plate, then dissected into 1 mm^2 large pieces, and maintained in a CO_2 incubator with 10% MEM. After 24 h, 2 mL 10% MEM was added and then changed every 36 h. The sections of tumor were not removed until tumor cells grew in the plate. Tumor cells were expanded in culture and designated as "N".

Genomic DNA, Total RNA Extraction

DNA and total RNA were extracted from 16HBE cells, transformed cells, "SA" cells, "N" cells and NCIH460 cell line (a large cell lung cancer cell line, provided by Dr. LIU, that lacks FHIT expression and Fhit protein[11]) using TRIZol (Invitrogen) as described by the manufacturer.

RT-PCR Amplification of the FHIT and P16 Genes and Sequencing Analysis of a FHIT Gene Aberrant Transcript

Total RNA from each cell line was reverse transcribed using Themscript™ RT-PCR kits (Invitrogen). Using this cDNA as a template, PCR amplifications of the FHIT gene and the P16 gene were performed, using primers for FHIT exons 1-9 and P16 exons 2-3 (Table 1) in 50 μL final volume with 2 μL of cDNA, 5 μL of 10×PCR buffer, 1 μL of 10 μmol/L dNTP, 1 μL of 10 μmol/L primer, and 2 units of Tag polymerase. The amplifications were performed in an Ependorf 5330 Mastercycler for 35 cycles at 94℃ for 30 s (for denaturation), at 53℃-

60℃ (varied for specific primer pairs) for 30 s (for annealing), at 72℃ for 45 s (for extension), then a final elongation for 5 min at 72℃. For FHIT gene amplifications, PCR reactions were carried out for either 35 cycles or 25 cycles, and PCR products from 25 cycles were used for second or nested PCR amplification. The integrity of cDNAs used in the present study was checked with PCR using β-action specific primers (Table 1). PCR amplification of no cDNA served as a blank control. Two μL of diluted (20-fold) first-step PCR products of FHIT gene cDNA (25 cycles) was amplified in 50 μL of the second PCR mixture under the same conditions as those for the first PCR, except for the primers and cycles (30 cycles). Ten μL of the PCR products was analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide, and one of those products demonstrating altered band size was sequenced.

TABLE 1

PCR Primers for cDNA

Fragment	Sequence (5′-3′)	Size (bp)
FHIT cDNA		
Exon 1-9	F CTGCTCTGTCCGGTCACA	802
	R CTGTGTCACTGAAAGTAGACC	
Exon 1-4	F CTGCTCTGTCCGGTCACA	336
	R TTTCAGAAGACTGCTACCTCTT	
Exon 5-9	F TGAGGACATGTCGTTCAGATTTGG	456
	R CTGTGTCACTGAAAGTAGACC	
P16 Cdna		
Exon 2-3	F ATGATGATGGGCAGCGCC	350
	R CGAGGTTTCTCAGAGCCT	
β-actin	F CGTCTGGACCTGGCTGGCCGCGACC	588
	R CTAGAAGCATTTGCGGTGGACGATG	

Note. F: forward; R: reverse.

PCR Amplifications of FHIT Gene Exons 5-8 and P16 exon 2 and SSCP Analysis

PCR amplifications were carried out using genomic DNAs and the primer pairs (Table 2). The amplification consisted of 35 cycles (at 94℃ for 30 s, at 53℃-58℃ for 30 s, and at 72℃ for 45 s) and a final elongation for 5 min at 72℃. SSCP was performed as described previously[12]. Eight μL of PCR product was mixed with 8 μL of loading buffer (95% formamide, 10 mmol/L EDTA 0.05% bromphend blue, 0.05% xylene cyanol FF), denatured at 98℃ for 10 min, and then cooled on ice for 5 min. Samples were electrophoresed and detected on 6%-8% non-denaturing polyacrylamide gels both in the presence and absence of 10% glycerol at 20℃ 120 V for 4-5 h.

TABLE 2

PCR Primers for Genomic DNA

Fragment	Sequence (5′-3′)	Size (bp)
FHIT		
Exon 5	F ATGGCATCCTCTCTGCAA	302
	R TTCATTTGGCTGGTTAGG	
Exon 6	F ATGTCCTTGTGTGCCCGCT	145
	R CTGCATGGAAAAGGTGAGAGA	
Exon 7	F TGGTCCCCATGAGAATACTA	310
	R TTACGGCTCTAACACTGAGG	
Exon 8	F GGAGTAATTGGGCTTCAT	200
	R AGGTTGATGTCATCCCAC	
P16		
Exon 2	F TGTTCTCTGGCAGGTCAT	342
	R CTCAGATCATCAGTCCTCAC	

Note. F: forward; R: reverse.

Western Blot Analysis

Cultured cells were directly lysed (2×10^7 cells/mL). Proteins (50 μg/lane) were fractionated by SDS-PAGE in a 15% gel, electroblotted onto PVDF membrane (2.5 mA/cm^2 for 30 min) and reacted with anti-GST-Fhit antibody at 1:5000 dilution according to standard procedures, using goat anti-rabbit horseradish peroxidase-conjugated immunoglobulin for detection of the primary antibody. The CST Western detection system was used, and the blot was exposed to X-ray film for 1-60 min.

RESULTS

Cytotoxicity of Crystalline NiS

In initial studies, we tested the toxicity of NiS to 16HBE cells at concentrations ranging from 0.05 μg/cm^2 to 2.00 μg/cm^2 (Table 3). Colony formation efficiencies (CEF) and relative colony efficiencies (RCE) of cells showed an inverse dose responsive relationship with NiS concentration. We selected 0.25-2.00 μg/cm^2 concentrations of NiS to study transformation by NiS.

Transformation of 16HBE by Crystalline NiS

After 6 times of treatment, alterations in morphology and growth pattern were observed in the

TABLE 3

Effect of NiS on Colony Formation Efficiency of 16HBE

Dose of NiS (μg/cm^2)	CFE (%, $\bar{x} \pm s$)	RCE (%)
0.00	49.3±2.5	100.0
0.05	47.7±1.5	96.8
0.25	42.3±2.5	85.8
0.50	25.0±3.5	50.7
1.00	17.7±2.1	35.9
2.00	6.0±1.0	12.2

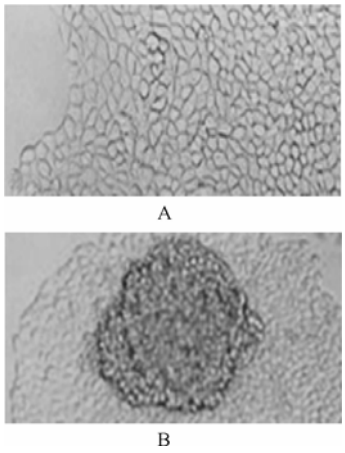

FIG. 1. Morphology of 16HBE cells and NiS-transformed cells. Note that Negative control 16HBE cells (A) grew in a monolayer manner, NiS-transformed cells (B) exhibited overlapping growth (×200).

TABLE 4

Colony Formation Efficiency of Transformed Cells in Soft Agar

Time	Group	Dose (μg/cm^2)	Plate No.	CFE (‰, $\bar{x} \pm s$)
24 h	Negative Control	0.00	3	0.33±0.58
	Low Dose	0.25	3	2.67±0.58*
	Middle Dose	1.00	3	4.67±0.58*
	High Dose	2.00	3	7.00±1.00*
	Positive Control (μmol/L)	2.00	3	13.00±2.65*
48 h	Negative Control	0.00	3	0.33±0.58
	Low Dose	0.25	3	3.33±0.58*
	Middle Dose	1.00	3	7.00±2.00*
	High Dose	2.00	3	12.33±1.53*
	Positive Control (μmol/L)	2.00	3	17.67±2.08*

Note. Negative control: tristilled water. Positive control: anti-BPDE. U test, *P<0.01, compared with negative control group.

NiS-treated cells. NiS-treated cells exhibited overlapping growth, whereas negative control cells grew in a monolayer (Fig. 1). We plated the control untreated cells and NiS-transformed cells in soft-agar and monitored their anchorage independent growth. As compared with negative control cells, colony formation efficiency of the transformed cells in soft agar showed significant increases (P<0.01) in a dose-dependent manner (Table 4).

Tumor Growth in Nude Mice

Tumor formation in nude mice by injected transformed cells could reflect the tumorigenic phenotype.

Three weeks after cells were injected, all the animals injected with NiS-transformed cells and "SA" cells exhibited tumor formation. Sections of tumor tissues from multiple areas were examined histopathologically and a squamous cell carcinoma was confirmed (Fig. 2).

FIG. 2. Tumor in athymic nude mice induced by injected transformed cells.

Expression of P16 Gene

RT-PCR was used to examine mRNA expression of the P16 gene and β-actin gene in negative control cells, NiS-transformed cells, "SA" cells and "N" cells. All the cells exhibited normal mRNA expression of the β-actin gene and the P16 gene (Fig. 3).

Expression of FHIT Gene and Fhit Protein

No product was observed in all the cell lines from the first PCR amplification of the FHIT gene exons 1-9. After the second PCR amplification, either aberrant transcripts or FHIT gene expression loss was observed in all the cell lines except for the negative control cell line. Both normal and aberrant transcripts were observed in NiS-transformed cells and "N" cells, while only aberrant transcripts were found in "SA" cells. By nested RT-PCR amplification of FHIT exons 1-9, aberrant transcripts were only observed in "SA" cells compared with the negative control cell line. Loss of FHIT gene expression was observed in the NCIH460 cell line (Fig. 4).

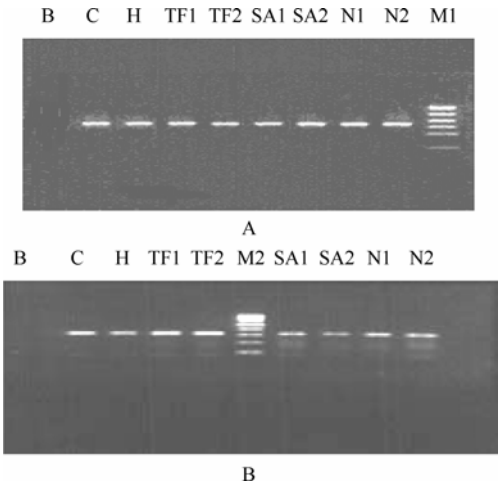

FIG. 3. RT-PCR analysis of β-actin (A) and P16 gene (B). M1: PCR Marker(1543 bp, 994 bp, 695 bp, 515 bp, 377 bp, 237 bp); C: negative control cell line; TF: transformed cell line; SA: "SA" cell line; N: "N" cell line; H: NCIH460; M2: 100 bp DNA ladder; B: blank control.

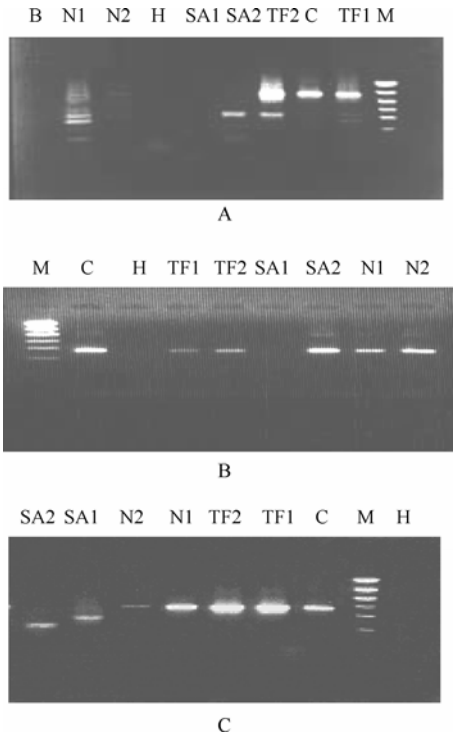

FIG. 4. RT-PCR analysis of FHIT gene exons1-9(A), FHIT gene exons1-4(B)and FHIT gene exons5-9(C). M: PCR Marker; C: negative control cell line; TF: transformed cell line; SA: "SA" cell line; N: "N" cell line; H: NCIH460. B: blank control.

Compared to the negative control cell line, loss of expression of the Fhit protein was observed in transformed cells "SA" cells, and "N" cells by using Western blot analysis (Fig. 5).

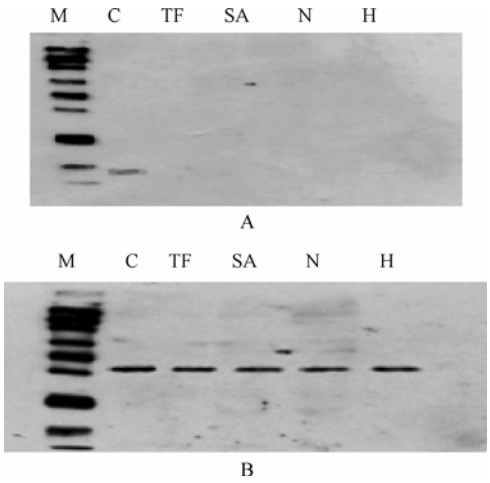

FIG. 5. Western blot analysis of Fhit protein(A) and β-actin protein(B). M: Protein Marker(200kD, 100kD, 80kD, 60kD, 50kD, 40kD, 30kD, 20kD, 10kD); C: negative control cell line; TF: transformed cell line; SA: "SA" cell line; N: "N" cell line; H: NCIH460.

Sequencing Analysis

Sequencing confirmed that one of the aberrant transcripts of the FHIT gene observed in the "SA" cell line contained the deletion of exons 6-8 and the insertion of 36 bp sequence replacing exons 6-8 (Figs. 6 and 7).

PCR-SSCP Analysis

No mutation of exon 2 or exons 2-3 in the P16 gene or FHIT exons 5-8, and exons 1-4 or exons 5-9 was observed in transformed cell lines, "SA" cell lines or "N" cell lines by PCR-SSCP analysis.

DISCUSSION

Neoplastic transformation of cells in culture is one of the few systems that can be used to study the mechanisms of human carcinogenesis. To date most of the neoplastic transformation studies have been carried out using rodent or human fibroblasts. Since most cancers originate from epithelial cells, neoplastic transformation of human epithelial cells can provide a powerful model for studies of carcinogenesis *in vitro*.

In this study, we selected a human bronchial epithelial cell line (16HBE) immortalized by SV40 large T antigen to study neoplastic transformation by crystalline nickel sulfide. 16HBE is an immortal cell line[13], which grows at high cell density, forms minute colonies in soft agar, and does not induce tumors when injected into nude mice. Therefore, this cell line is a useful target to study neoplastic transformation.

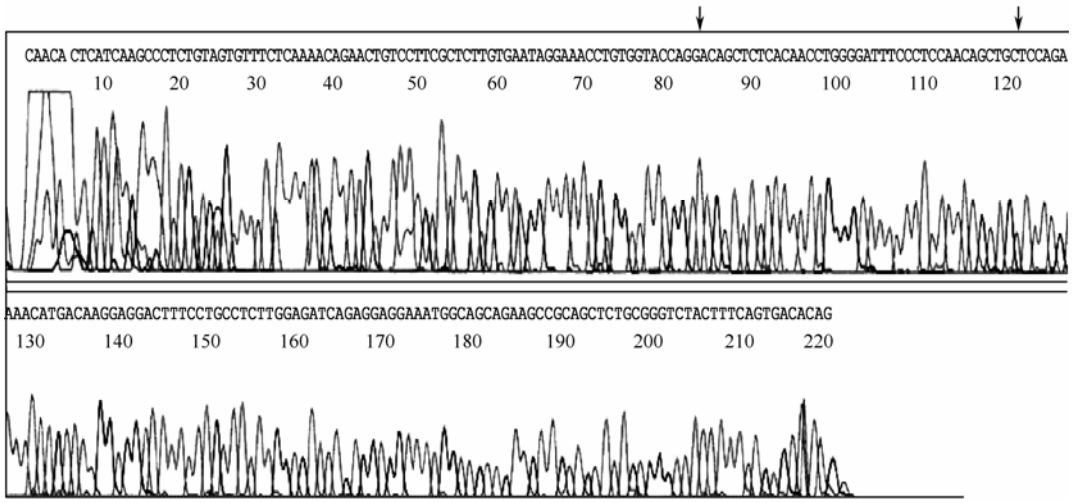

FIG. 6. Sequencing analysis of FHIT gene aberrant transcript. Note that arrows indicate the junctions between exon 5 and exon 9.

1. TGAGGACATGTCGTTCAGATTTGGCCAACATCTCATCAAGCCCTCTGTAGTGTTTCTCAAA

2. **********CAACATCTCATCAAGCCCTCTGTAGTGTTTCTCAAA

1. CAGAACTGTCCTTCGCTCTTGTGAATAGGAAACCTGTGGTACCAGGAC**ATGTCCTTGTGT**

2. CAGAACTGTCCTTCGCTCTTGTGAATAGGAAACCTGTGGTACCAGGAC----------

1. GCCCGCTGCGGCCAGTGGAGCGCTTCCATGACCTGCGTCCTGATGAAGTGGCCGATT

2. ----------

1. TGTTTCAGACGACCCAGAGAGTCGGGACAGTGGTGGAAAAACATTTCCATGGGACCT

2. ----------

1. CTCTCACCTTTTCCATGCAGGATGGCCCCGAAGCCGGACAGACTGTGAAGCACGTTC

2. ----------

1. ACGTCCATGTTCTTCCCAGGAAGGCTGGAGACTTTCACAGGAATGACAGCATCTATG

2. ----------

1. AGGAG++CTCCAGAAACATGACAAGGAG

2. ----------AGCTCTCACAACCTGGGGATTTCCCTCCAACAGCTGCTCCAGAAACATGACAAGGAG

1. GACTTTCCTGCCTCTTGGAGATCAGAGGAGGAAATGGCAGCAGAAGCCGCAGCTCTGCGG

2. GACTTTCCTGCCTCTTGGAGATCAGAGGAGGAAATGGCAGCAGAAGCCGCAGCTCTGCGG

1. GTCTACTTTCAGTGACACAG

2. GTCTACTTTCAGTGACACAG

FIG. 7. Comparison between normal transcript and aberrant transcript of FHIT exon 5-9. 1: normal transcript (456 bp); 2: aberrant transcript (247 bp). *: primer of sequencing; ---: deleted base; +: inserted base.

Carcinogenesis is a multistage and multifactor process that progressively converts normal cells into malignant cancer cells. In general, cultured human cells are more resistant than rodent cells to neoplastic transformation by carcinogens[8]. In the present study, after 6 rounds of treatment with crystalline NiS, the 35th passage cells exhibited overlapping growth. Compared with negative control cells, colony formation efficiency of NiS-treated cells in soft agar showed a significant increase (P<0.01) in a time- and dose-dependent manner. NiS-treated cells could form a tumor in nude mice. The tumor was a poorly differentiated squamous cell carcinoma in morphology confirmed by histopathological examination, suggesting that crystalline NiS can strongly induce neoplastic transformation of 16HBE

cells. The transformed model *in vitro* provides a potential tool for studying the molecular mechanism of nickel carcinogenesis.

Frequent allelic loss was observed on chromosome 3p in lung cancer and preneoplastic bronchial lesions, indicating that inactivation of putative tumor suppressor genes on 3p may be involved in early steps of lung carcinogenesis. Ohta *et al.*[14] have identified the FHIT gene on 3p14.2, spanning the FRA3B common fragile site and the t (3:8) break point associated with hereditary renal cell carcinomas. The FHIT gene is more than 1 Mb in size, encodes a 1.1-kb cDNA with 10 small exons and a cytoplasmic M_r 16 800 protein with diadenosine triphosphate (Ap3A) hydrolase activity[15-16]. Previous studies have shown that the FHIT gene is inactivated by deletions in carcinoma cell lines and primary tumors of the lung, head and neck, breast, stomach, colon, and several other tissues, sequencing of nearly 900 kb of the gene has led to precise definition of biallelic deletions within FHIT in representative cancer-derived cell lines[17-19]. In addition, it was reported that FHIT expression is correlated with smoking or alcohol drinking[20-22]. In the present study, no PCR product was found in all the cell lines following the first PCR amplification of FHIT exons 1-9, but after the second PCR or nested PCR amplification, the product of FHIT gene expression was detected, suggesting that these cell lines express very low levels of FHIT mRNA, which is in agreement with the reports of others in cancer cell lines or cancer tissues[14,23-24]. Therefore, it is necessary to detect the alterations of FHIT mRNA by the second PCR or nested PCR amplification. No mutation of FHIT exons 5-8 or FHIT exons 1-9 was observed in transformed cell lines, “SA” and “N” cell lines. In contrast, aberrant transcripts or FHIT gene expression loss and Fhit protein loss were observed in transformed cell lines, “SA” and “N” cell lines. These data suggest that the loss and rearrangement of the FHIT gene derived from FRA3B breaks or gaps in the large FHIT intronic region induced by nickel, may give rise to cDNA splicing aberrants and result in loss of Fhit protein.

In this study, both normal and aberrantly sized products were observed in the transformed cell line. Sozzi *et al.*[25] have found abnormal sized transcripts in lung cancers with normal transcript, which can reflect contaminating normal cells within the tumors. We also found both normal transcript and abnormal transcripts in “N” cell line, but only abnormal transcripts or FHIT expression loss in “SA” cell lines, suggesting that simultaneous presence of normal FHIT transcripts as well as abnormal FHIT transcripts in transformed cell lines and “N” cell lines is not only attributed to normal cell contamination but also related to tumor heterogeneity. The occurrence of normal and aberrant transcripts in the same cell lines might be explained by the presence of several distinct cell populations, which would obscure the detection of homozygous deletions in a subpopulation. Since the “SA” cell line is a pure population of malignant transformed cells generated by subcloning transformed cells, it only expresses abnormal FHIT transcripts or loses FHIT expression. The presence of various types of aberrant transcripts indicates the complexity and heterozygosity of FHIT gene lesions. Sequencing analysis of a shorter transcript revealed lack of exons 6-8 and insertion of 36 bp sequence replacing exons 6-8, further confirming the loss of FHIT exons induced by crystalline NiS. Western blot analysis showed that Fhit protein was undetectable in transformed cell lines, “SA” and “N” cell lines. Furthermore, lack of detectable Fhit protein in cell lines was correlated with FHIT gene deletions and altered mRNA expression.

During neoplastic transformation of 16HBE cells induced by crystalline NiS, no alteration of the P16 gene was observed, suggesting that the P16 gene is not involved in nickel carcinogenesis.

In conclusion, the FHIT gene rather than the P16 gene plays a definite role in nickel carcinogenesis. Abnormalities of the FHIT gene forges a link between chromosome fragile sites and many cancers[26]. Thus, alterations of the FHIT gene induced by crystalline NiS may be a molecular event associated with carcinogenesis, chromosome fragile site instability and neoplastic cell transformation. The FHIT gene may be one of the important target genes altered by exotic carcinogens. Further molecular analysis and additional functional studies of the FHIT gene are required to facilitate better understanding of the molecular mechanism of xenobiotic carcinogenesis.

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REFERENCES

1. IARC International Agency for Research on Cancer (1990). Monographs on the Evaluation of Carcinogenic Risk to Humans, Vol. 49, Chromium, Nickel and Welding, IARC, Lyon.
2. Oller A R, Costa M, and Oberdorster G (1997). Carcinogenicity assessment selected nickel compounds. *Toxicol Appl Pharmacol* **143**, 152-166.
3. Dunnick J K, Elwell M R, Radovsky A E, *et al*. (1995).

Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. *Cancer Res.* **55**, 5251-5256.
4. Costa M, Mollenhauer H H (1980). Carcinogenic activity of particulate nickel compounds is proportional to their cellular uptake. *Science* **209**, 515-517.
5. Evans R M, Davies P J, Costa M (1982). Video time-lapse microscopy of phagocytosis and intercellular fate of crystalline nickel sulfide particles in cultured mammalian cells. *Cancer Res* **42**, 2729-2735.
6. Coogen T P, Latta D M, Snow E T, *et al*. (1989). Toxicity and carcinogenicity of nickel compounds. *Crit Rev Toxicol* **19**, 341-384.
7. Rani A S, Qu D Q, Sidhu M K, *et al*. (1993). Transformation of immortal, non-tumorigenic osteoblast-like human osteosarcoma cell to the tumorigenic phenotype by nickel sulfate. *Carcinogenesis* **14**, 947-953.
8. Lin X H, Costa M (1994).Transformation of human osteoblasts to anchorage-independent growth by insoluble nickel particles. *Environ Health Perspect* **102**(suppl 3), 289-292 .
9. Fletcher G G, Rosetto F E, Turnbull J D, *et al*. (1994). Toxicity, uptake and mutagenicity of particulate and soluble nickel compounds. *Environ Health Perspect* **102**(suppl 3), 69-79.
10. Costa M, Yan Y, Zhao D, *et al*. (2003). Molecular mechanisms of nickel carcinogenesis: gene silencing by nickel delivery to the nucleus and gene activation/inactivation by nickel-induced cell signaling. *J Environ Monit* **5**, 222-223
11. Sard L, Accomero P, Tomielli S, *et al*. (1999). The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. *Proc Natl Acad Sci U S A* **96**, 8489-8492.
12. Ji W D, Wu Z L, Chen J K (2001). Study on the mutation of p53 tumor suppressor gene in human bronchial epitheial cell line treated with trans-BPDE. *Chin Occup Med* **28**, 12-13. (In Chinese)
13. 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**, 38-47.
14. Ohta M, Inoue H, Cotticelli M G, *et al*. (1996). The FHIT gene, Spanning the chromosome 3P14.2 fragile site and renal carcinoma-associated t (3;8) breakpoint, is abnormal in digestive tract cancers. *Cell* **84**, 587-597.
15. Blackburn G M, Liu X, Rosler A, *et al*. (1998). Two hydrolase resistant analogues of diadenosine 5', 5''' P1- P3 triphosphate for studies with Fhit, the human fragile histidine triad protein. *Nucleotides* **17**, 301-308.
16. Murphy G A, Halliday D, Mclennan A G (2000). The Fhit tumor suppressor protein regulates the intracellular concentration of diadenosine triphosphate but not diadenosine tetraphate. *Cancer Res* **60**, 2342-2344.
17. Buttitta F, Marchetti A, Radi O, *et al*. (1998). Evaluation of FHIT gene alterations in ovarian cancer. *Br J Cancer* **77**, 1048-1051.
18. Yuan B Z, Keck-Waggoner C, Zimonjic D B, *et al*. (2000). Alterations of the FHIT gene in human hepatocellular carcinoma. *Cancer Res* **60,** 1049-1053.
19. Druck T, Berk L, Huebner K (1999). FHITness and cancer. *Oncology Res* **10**, 341-345.
20. Nelson H H, Wiencke J K, Gunn L, *et al*. (1998). Chromosome 3P14.2 alterations in lung cancer: evidence that FHIT exon deletion is a target of tobacco carcinogens and asbestos. *Cancer Res* **58**, 1804-1807.
21. Pylkkanen L, Wolff H, Stjernvall T, *et al*. (2002). Reduced Fhit protein expression and loss of heterozygosity at FHIT gene in tumours from smoking and asbestos-exposed lung cancer patients. *Int J Oncol* **20**, 285-290.
22. Meninc C, Santacatterina M, Zambon A, *et al*. (2000). Anomalous transcripts and allelic deletions of the FHIT gene in human esophageal cancer. *Cancer Genet Cytogenet* **119**, 56-61.
23. Gramantieri L, Chieco P, Ditomaso M, *et al*. (1999). Aberrant fragile histidine triad gene transcripts in primary hepatocellular carcinoma and liver cirrhosis. *Clin Cancer Res* **5**, 3468-3475.
24. Greenspan D L, Connolly D C, Wu R, *et al*. (1997). Loss of FHIT expression in cervical carcinoma cell lines and primary tumors. *Cancer Res* **57**, 4692-4698.
25. Sozzi G, Veronese M L, Negrini M, *et al*. (1996). The FHIT gene at 3P14.2 is abnormal in lung cancer. *Cell* **85**, 17-26.
26. Penisi E (1996). New gene forges link between fragile site and many cancer. *Science* **272**, 649.

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