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Carcinogenic potential and genomic instability of beryllium sulphate in BALB/c-3T3 cells

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Abstract

Occupational exposure to beryllium (Be) and Be compounds occurs in a wide range of industrial processes. A large number of workers are potentially exposed to this metal during manufacturing and processing, so there is a concern regarding the potential carcinogenic hazard of Be. Studies were performed to determine the carcinogenic potential of beryllium sulfate ($BeSO_4$) in cultured mammalian cells. BALB/c-3T3 cells were treated with varying concentrations of $BeSO_4$ for 72 h and the transformation frequency was determined after 4 weeks of culturing. Concentrations from 50–200 μg $BeSO_4$/ml, caused a concentration-dependent increase (9–41 fold) in transformation frequency. Non-transformed BALB/c-3T3 cells and cells from transformed foci induced by $BeSO_4$ were injected into both axillary regions of nude mice. All ten Be-induced transformed cell lines injected into nude mice produced fibrosarcomas within 50 days after cell injection. No tumors were found in nude mice receiving non-transformed BALB/c-3T3 cells 90 days post-injection. Gene amplification was investigated in K-*ras*, c-*myc*, c-*fos*, c-*jun*, c-*sis*, *erb-B2* and *p53* using differential PCR while random amplified polymorphic DNA fingerprinting was employed to detect genomic instability. Gene amplification was found in K-*ras* and c-*jun*, however no change in gene expression or protein level was observed in any of the genes by Western blotting. Five of the 10 transformed cell lines showed genetic instability using different random primers. In conclusion, these results indicate that $BeSO_4$ is capable of inducing morphological cell transformation in mammalian cells and that transformed cells induced by $BeSO_4$ are potentially tumorigenic. Also, cell transformation induced by $BeSO_4$ may be attributed, in part, to the gene amplification of K-*ras* and c-*jun* and some $BeSO_4$-induced transformed cells possess neoplastic potential resulting from genomic instability. (Mol Cell Biochem **222**: 69–76, 2001)

Key words: cell transformation, tumorigenicity, genomic instability, beryllium sulphate

Introduction

Beryllium (Be) is widely used in industries due to its special properties, such as being lighter than aluminum yet 40% more rigid than steel [1]. Although a relatively small number of workers are potentially exposed to high levels of Be in the refining and machining of the metal and in production of Be-containing products, a growing number of workers are exposed to lower levels of Be in ceramics, production of dental equipment and supplies, golf club manufacturing, soldering and welding as well in the electronics, aircraft, aerospace and nuclear industries [2].

Beryllium is classified as a potential carcinogen in humans and inhaled beryllium causes a high incidence of rat lung tumors. Their mutagenicity and carcinogenicity have been extensively studied in the laboratory and in exposed workers [2–4]. Bacterial cells seem insensitive to Be, and most results from bacterial mutagenicity assay systems have been negative [5–8]. In mammalian cells, however, Be induced sister chromatid exchange (SCE), chromosomal aberrations

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(CA), *HPRT* gene mutation and cell transformation [7, 9–13]. The carcinogenicity of Be has been demonstrated in several animal species including rats, mice, rabbits and monkeys [2–5, 14]. A report from the International Agency for Research on Cancer [2] concluded that there is sufficient evidence in humans of the carcinogenicity of Be and Be-compounds, however, little is known regarding the mechanism of Be carcinogenesis.

Morphological cell transformation is a useful short-term test for the *in vitro* detection of potential carcinogens [15]. The athymic nude mouse is an excellent animal model for determining the tumorigenesis of morphologically transformed cells [16]. Cell transformation coupled with the nude mouse/tumorigenesis assay is a useful approach to study the carcinogenic potential of environmentally and occupationally related agents. Moreover, it enables the study of mechanistic and/or sequential processes during carcinogenesis. It is known that genetic alterations including gene amplification, deletion, and point mutation may play an important role in the activation and/or inactivation of cancer-related genes. In this investigation, an effort has been made to sequentially study the morphological transforming activity of $BeSO_4$ in BALB/c-3T3 cells and to determine whether these transformed cells are capable of producing tumors in the nude mouse assay system. Also, the possible mechanism of Be-induced cell transformation related to the amplification and/or expression of cancer-related genes was investigated. In addition, genomic instability in these transformed cells was also studied.

Materials and methods

Chemicals and reagents

Beryllium sulfate tetrahydrate ($BeSO_4 \cdot 4H_2O$, 99.99% pure) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA) and was freshly made and diluted into different concentrations with sterile water. Eagle's minimum essential medium (MEM), fetal bovine serum (FBS), L-glutamine, trypsin, and penicillin-streptomycin were purchased from Sigma (St. Louis, MO, USA).

Cell culture

BALB/c-3T3 clone A31-1-13 cells, kindly provided by Dr. M.A. Cifone (Covance Laboratories Inc., Vienna, VA, USA), were used in this study. The cells were maintained in MEM supplemented with 10% FBS, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin and incubated at 37°C with 5% CO_2. The cultures were split before reaching 70% confluence.

Cytotoxicity assay

Exponentially growing cells were seeded into 25 cm^2 flasks at a density of 3×10^4 cells/5 ml of complete medium. After 24 h culturing, the cells were exposed to final concentrations of $BeSO_4$ from 50–200 µg/ml for 72 h. The viable cells were counted under a microscope using trypan blue exclusion assay. The doses for the transformation assay were chosen from a range of 30–90% relative to cell survival.

Transformation assay

BALB/c-3T3 cells (3×10^4) at passage 5–8 were seeded into 25 cm^2 flasks (at least 25 flasks for each group) with 5 ml medium. Exposure was initiated 24 h later with final concentrations of 0, 50, 100, 200 µg $BeSO_4$ /ml for 72 h. After treatment the cells were rinsed 3 times with phosphate buffered saline (PBS) and replenished with fresh maintenance medium (7.5% FBS). The cultures were re-fed twice a week for an expression period of 4 more weeks. At the end of the expression period, cells from foci that were larger than 4 mm in diameter with piling characteristics were isolated and established as transformed cell lines for tumorigenicity testing in nude mice. After isolation, the flasks were washed with PBS, then fixed with methanol and stained with 10% Giemsa. Only Type 3 foci [17] in both control and treatment groups were scored as transformed foci.

Tumorigenicity in athymic nude mice

Athymic female nude mice (nu/nu, 3–4 weeks old) and heterozygotic female mice (+/nu) were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA). Thirty-six nude mice were divided randomly into eleven groups (three mice/group, six mice in the BALB/c-3T3 control group). The transformed cell lines were passed 4–6 times prior to injection. Ten transformed cell lines and a non-transformed BALB/c-3T3 cell line were separately harvested and concentrated to densities of 10^7 cells/ml of medium. Both sites of the axillary region of the nude mice were subcutaneously injected with 0.1 ml of cell suspensions (1×10^6 cells/each site). The nude mice were maintained under sterile conditions according to protocol from the American Association for the Accreditation of Laboratory Animal Care. Autoclaved cages, drinking water and food were changed once a week. Four heterozygotic mice were used as room and bedding controls for serosurveillance of rodent pathogens (Complete Health Monitoring Profile, Charles River Laboratories, Wilmington, MA, USA).

The mice were screened twice a week for the appearance and size of tumors. When tumor size at one site reached 1.5–

2.0 cm in length or width, a mouse was euthanized using sodium pentabarbitol. The tumors were surgically removed under sterile conditions and weighed, then divided into three parts. One portion was used for cell culturing, one piece was fixed in 10% neutral buffered formalin (NBF) for histopathological studies, and the third portion was frozen in liquid nitrogen for future analysis. If no visible tumor was found three months after injection, the skin around the injection site was removed and then examined histopathologically. The lungs were also collected for histopathological monitoring of tumor metastases. The tumor, skin or lung tissue, was fixed with 10% NBF and sectioned onto slides. The slides were then stained with hemotoxylin and eosin. Blind assessment was used for the histopathological analysis.

Differential PCR

DNA was isolated from 10 different Be-transformed cell lines and a non-transformed BALB/c-3T3 cells according to standard protocol [18]. Differential PCR was carried out independently using primers for K-*ras*, c-*fos*, c-*jun*, c-*myc*, c-*sis*, *erb-B2* and *p53* (Table 1). Briefly, template DNA (200 ng), a target gene primer pair (30 pmol each), reference gene (β-actin, 10–30 pmol), dNTPs (0.2 mM) and *Taq* polymerase (1.25 U, Promega, Madison, WI, USA) were added in PCR buffer and run for 30 cycles (denaturation at 94°C for 1 min; annealing at 50–59°C for 1 min; and extension at 72°C for 2 min). Following the PCR reaction, products were electrophoresed on a 2% agarose gel and the images were analyzed using the Eagle Eye II (Stratagene, La Jolla, CA, USA) gel documentation system. NIH Image for Macintosh (NIH, Bethesda, MD, USA) was used to determine the intensities of the resulting bands. The relative ratio of the intensity of the target gene to that of the reference gene was calculated allowing the gene copy number to be estimated.

RAPD analysis

Six arbitrary primers were used for randomly amplified polymorphic DNA (RAPD) analysis. The sequences of these primers are shown in Table 2. DNAs (0.5 μg) from each cell line (non-transformed and transformed BALB/c-3T3 cells) were amplified with each arbitrary primer (50 pmol), *Taq* DNA polymerase (1.25 U; Promega, Madison, WI, USA), dNTPs (100 μM; Promega) and $MgCl_2$ (2.5 mM) in 50 μl of reaction buffer for 40 cycles. PCR conditions were: denaturing at 94°C for 30 sec, annealing at 40°C for 1 min and extension at 72°C for 1 min. PCR products (10 μl each) mixed with loading buffer were loaded on 2% agarose gels and electrophoresed with 100 V for 1 h. The gels were stained with ethidium bromide (0.5 μg/ml), visualized using an Eagle Eye II documentation system and photographed. The banding profiles of non-transformed and transformed cell lines were analyzed based on changes in the intensity of a band, band shifts, missing or new bands.

Table 1. Primer sequences for proto-oncogenes and tumor suppressor gene used in differential PCR studies

Gene	Primer sequence
c-*jun*	5′-AACTCGGACCTTCTCACGTCG-3′ 3′-TGCTGAGGTTGGCGTAGACC-5′
K-*ras*	5′-TCTGTTCGTGCAAACTGTCAGC-3′ 3′-TGTCTGCCTACGCCTCTGAAAG-5′
c-*fos*	5′-CGTTGCAGACTGAGATTGCC-3′ 3′-ACCGGACAGGTCCACATCTG-5′
c-*myc*	5′-TCCATTCCGAGGCCACAGCAAG-3′ 3′-TCAGCTCGTTCCTCCTCTGACG-5′
c-*sis*	5′-GGTTGAGTGTCCCAGCATG-3′ 3′-TGGCCACTAGGATGTGCTAC-5′
erb-B2	5′-ATGCAAAGTGCAATGAAGACC-3′ 3′-TGTTCCTCCCTTTGTACTGTCC-5′
p53	5′-CATCACCTCACTGCATGGACG-3′ 3′-CAGACTTGGCTGTCCCAGACTG-5′
β-actin	5′-AGGCATTGTGATGGACTCCG-3′ 3′-AGTGATGACCTGGCCGTCAG-5′

Western blot analysis

Protein was obtained from 10 Be-transformed and one non-transformed BALB/c3T3 cell lines by standard methods [18]. Five micrograms of each protein sample was mixed with an equal volume of 2X SDS sample loading buffer containing 5% 2-mercaptoethanol and heated to 95°C for 3 min before being subjected to Western blot analysis.

Western blotting was carried out according to an established method [19]. Briefly, samples were loaded on a 5–17%

Table 2. Primers used for RAPD analysis to detect DNA alterations in non-transformed, $BeSO_4$-transformed cell lines

Serial No.	Primer No.	Sequence (5′ – 3′)
1	448	TGGGCATCTG
2	453	AGCTGCCGGG
3	457	AAGGCTAGCG
4	460	AGGCATTCCC
5	485	CGGCCCCTGT
6	488	CAGGCCCTTC

polyacrylamide gradient gel, electrophoretically separated, and transferred to Immobilon PVDF transfer membranes. Primary antibodies were used to detect proteins produced by six different proto-oncogenes: *ras* (pan-*ras* Ab-3, Oncogene Research, Cambridge, MA, USA), K-*ras*, *sis*, *myc*, *erb*-B2 (Quality Biotech, Camden, NJ, USA), *fos* (Ab-1, Oncogene Research) and *jun* (Ab-1, Oncogene Research), and one tumor suppressor gene: *p53* (Ab-3, Oncogene Research). The antibody complexes were visualized using an avidin-alkaline phosphatase detection system. Resulting bands were analyzed using the Whole Band Analyzer (BioImage, Ann Arbor, MI, USA).

Statistical analysis

A *t*-test was used to compare the mean number of transformed foci/flask as well as tumor size or weight. Mean and standard deviations were calculated for all the data recorded. Transformation frequencies were also compared using the χ^2 test.

Results

As shown in Table 3, clear dose-dependent cytotoxicity was demonstrated, indicating that $BeSO_4$, at the concentrations tested, was toxic to BALB/c-3T3 cells. Compared with the control, the relative cell surviving rates decreased from 100 to 17.4% with increasing $BeSO_4$ concentrations from 0–200 µg/ml. Table 3 also shows a dose-response relationship for morphological transformation induced by $BeSO_4$. Transformation frequencies (TF) were 9, 14 and 41 fold greater than the spontaneous TF at doses of 50, 100 and 200 µg/ml, respectively. The mean numbers of transformed foci/flask were 8, 9 and 10 fold greater than that of the non-exposed control cells. The differences between control and test groups were statistically significant ($p < 0.001$).

In the tumorigenicity study, an animal was euthanized when tumor size reached 1.5–2.0 cm in length or width at one site. The earliest sacrifice of animals occurred only 12 days after injection with transformed cells (Fig. 1). The number of sacrificed animals increased rapidly from 10–87% between 12 and 34 days after inoculation with transformed cells. The remaining nude mice inoculated with transformed cells reached the tumor size limit within 50 days. In contrast, the six nude mice injected with non-transformed BALB/c-3T3 cells were kept for 90 days and no tumors were found.

The tumorigenic potential of transformed cells in nude mice is summarized in Table 4. All mice inoculated with transformed cells developed tumors, and tumors were seen at both sites except in one animal. The histopathological results showed that all tumors were fibrosarcomas, and no metastases were found in the lungs. Compared with non-transformed BALB/c-3T3 cells, tumor frequencies increased significantly and latency periods decreased. The tumor frequency, average tumor weight, average tumor size and the average latency period were not statistically different between transformed cell lines.

To further study the mechanism of $BeSO_4$-induced cell transformation, gene amplification, protein expression and genomic instability assays were carried out. Gene amplification was studied in proto-oncogenes (K-*ras*, c-*fos*, c-*jun*, c-*myc*, c-*sis*, *erb-B2*) and a tumor suppressor gene (*p53*) (Fig. 2). Increased copy number was found for c-*jun* and K-*ras*. None of the other genes tested showed amplification. In this study, analysis of gene expression was also conducted in all the genes as in amplification. Also, mutations in K-*ras*, codons 12, 13 and 61 were evaluated using the PCR-RFLP method. No increase in the expression in any of the genes were observed (data not shown) and no mutations were found in K-*ras*, codons 12, 13 or 61 in 10 $BeSO_4$-induced transformed cell lines (data not shown). In addition, Western blot analysis showed no difference in the levels of any of the proteins examined (K-RAS, MYC, FOS, JUN, SIS, ERB-B2 and P53) in Be-transformed cells.

Genomic changes in $BeSO_4$-induced transformed cell lines were detected by RAPD analysis (Table 5). Representative banding patterns of 10 different cell lines of $BeSO_4$-transformed cell DNA using primer #453 are shown in Fig. 3. Six random primers were used to randomly span the whole genome. Non-transformed BALB/c-3T3 cells and 10 transformed cell lines obtained from all three different concen-

Table 3. Cytotoxicity and morphological transformation induced by $BeSO_4$ in BALB/c-3T3 cells

$BeSO_4$ (µg/ml)	Flasks/ group	Type III foci	Mean no. of transformed foci flask[a] (× ± S.D.)	Relative cell surviving[b] (%, × ± S.D.)	Transformation frequency[c] (10^{-5} × ± S.D.)
0.0	54	18	0.33 ± 0.476	100.00	0.70 ± 0.57
50.0	60	156	2.60 ± 1.532*	68.20 ± 16.69	6.05 ± 0.78*
100.0	63	184	2.92 ± 1.619*	43.95 ± 8.13	10.08 ± 0.03*
200.0	72	245	3.40 ± 2.053*	17.40 ± 0.14	28.59 ± 4.82*

*Significantly different from control, $p < 0.001$. [a]Mean number of transformed foci/flask = total number of type III foci/number of flasks; [b]relative cell surviving = (number of live cells/number of live cells in control) × 100%; [c]transformation frequency = total number of type III foci/(total number of plated cells x relative cell surviving).

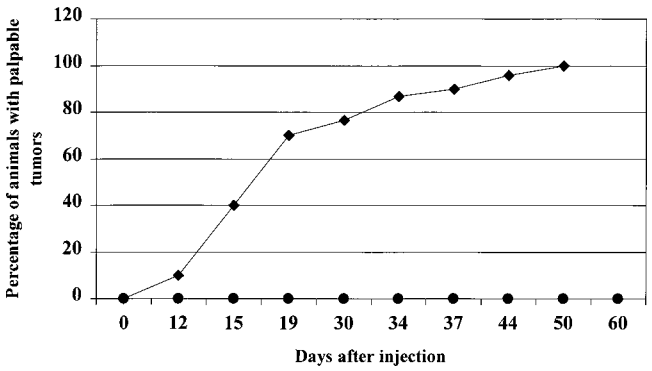

Fig. 1. Tumorigenic potential of Be-transformed cells. Mice injected with transformed cells (◆); mice injected with non-transformed BALB/c-3T3 cells (●).

trations were studied. Band shifts and changes in intensity were found in two transformed cell lines using primer 448. Similar changes including missing bands and the appearance of new bands were seen when primers 485 and 488 were used. Transformed cell lines 2, 3, 6, 8 and 10 also had genomic changes (Table 5).

Discussion

Beryllium sulfate is inactive in most bacterial mutagenesis assays. It induces mutations in neither *Salmonella typhimurium* (strains TA 98, 100, 1530, 1535, 1536, 1537, 1538 and LT_2), in the presence or absence of metabolizing systems, nor in *Escherichia coli* WP2 *uvr*A [5, 6, 20, 21]. Moreover, $BeSO_4$ was not cytotoxic in bacteria, and no growth inhibition was seen even with exposures up to 5 mg/plate with or without S9 metabolic activation in the Ames assay [5]. $BeSO_4$ was, however, cytotoxic to BALB/c-3T3 cells as shown in this study. It has been suggested that unlike mammalian cells, bacterial cells fail to uptake beryllium salts [2]. This seems to be a plausible explanation for the negative results from bacterial systems.

Exogenous metabolic activation was not used in this study, but a dose-response relationship in cell transformation induced by $BeSO_4$ was obtained, and the differences between the control and experimental groups were statistically significant ($p < 0.001$). These results indicate that additional metabolic activation was not necessary for $BeSO_4$ to induce morphological cell transformation in BALB/c-3T3 cells, but these results could not answer whether Be directly induced cell transformation without any metabolism, since BALB/c-3T3 cells still had metabolic capability, although it was much lower than in primary cells [17].

The mean numbers of transformed foci/plate and the transformation frequencies were used to evaluate the cell transformation assay [10, 17, 22–24]. For the former, because the cytotoxicity was not considered as a factor when simply counting the number of foci per plate, the transforming ability may not be accurately estimated. For example, in the current study, if the mean numbers of foci/flask were used to evaluate the transforming ability of $BeSO_4$, it would be only 8, 9 or 10 fold greater than that of the control. But the transformation frequencies were 9, 14 or 41 fold greater than

Table 4. Tumorigenic potential of transformed cells induced by $BeSO_4$ in nude mice

Transformed cells	No. of mice	Sites of inoculation	Characteristics of tumor					
			frequency	size (cm^3)	weight (g)	type	latency (days)	metastases
50.0 μg/ml								
C1	3	6	6	2.79 ± 0.46	1.49 ± 0.08	FS	37.3	0
C2	3	6	6	4.20 ± 0.62	2.09 ± 0.15	FS	15.0	0
C3	3	6	6	2.83 ± 0.49	1.53 ± 0.23	FS	19.0	0
Subtotal	9	18	18/18*	3.27 ± 0.83	1.72 ± 0.33		23.78 ± 10.71*	0
100 μg/ml								
C1	3	6	5	2.89 ± 1.22	1.45 ± 0.28	FS	43.7	0
C2	3	6	6	5.02 ± 1.09	1.83 ± 0.59	FS	12.0	0
C3	3	6	6	5.53 ± 2.92	3.34 ± 1.49	FS	19.0	0
C4	3	6	6	3.97 ± 0.87	2.24 ± 0.83	FS	17.7	0
Subtotal	12	24	23/24*	4.35 ± 1.82	2.22 ± 1.07		23.08 ± 13.05*	0
200 μg/ml								
C1	3	6	6	2.59 ± 1.05	1.34 ± 0.37	FS	16.3	0
C2	3	6	6	4.73 ± 2.35	2.28 ± 0.62	FS	31.3	0
C3	3	6	6	4.35 ± 1.36	1.70 ± 0.59	FS	15.0	0
Subtotal	9	18	18/18*	3.89 ± 1.76	1.77 ± 0.59		20.89 ± 8.02*	0
BALB/c 3T3 cells	6	12	0/12	0	0	–	> 90.00	0

FS – Fibrosarcoma. *Significantly different from control; C1, C2, C3, C4 are different transformed cell isolates from the same treatment group.

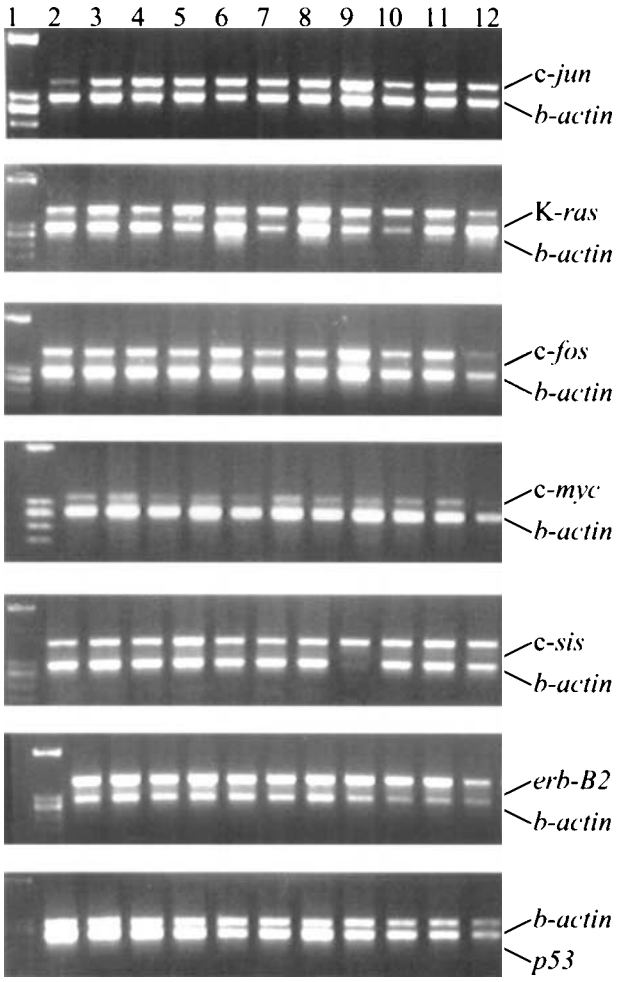

Fig. 2. Gene amplification for c-*jun*, K-*ras*, c-*fos*, c-*myc*, c-*sis*, *erb-B2* and *p53*. Lane 1: Φ174/*Hae III* molecular weight marker; lane 2: non-transformed BALB/c-3T3 cells; lanes 3–12: $BeSO_4$-transformed cells.

that of the control (Table 3). When cytotoxicity was included in the calculation, a much stronger transformation response was obtained at higher concentrations. Therefore, the mean number of foci/plate may not be a good indicator for evaluation of the transforming activity, particularly at toxic doses.

Carcinogenesis is thought to be a multistep process of loss of growth control arising in a single cell, including initiation, promotion and progression [25, 26]. Cell transformation *in vitro* also goes through a complex series of events with patterns of growth control changes similar to carcinogenesis *in vivo* [15, 27]. This relationship allows morphological cell transformation to be considered useful as an *in vitro* model for the investigation of chemical carcinogenesis [15, 28], however, its measured endpoint has an important drawback. Because morphologically transformed foci are not tumors, it cannot be clearly answered whether the morphologically altered foci have the potential of tumorigenesis *in vivo*. Therefore, it is very useful in understanding the carcinogenesis of

Table 5. Genetic instability detected in Be-transformed cell lines when compared to non- transformed BALB/c-3T3 cells

Primers	Genetic alterations in transformed cells lines									
	1	2	3	4	5	6	7	8	9	10
448	–	–	–	–	–	a,b	–	a	–	–
453	–	–	–	–	–	a,b,c	–	b,c	–	–
457	–	b,c	c	–	–	b	–	–	–	–
460	–	–	–	–	–	a,b	–	–	–	a,b
485	–	–	–	–	–	a,c	–	b	–	–
488	–	–	–	–	–	d,b	–	b	–	d,a

[a]band shift; [b]change in intensity; [c]missing band; [d]new band appearance.

chemicals when the cell transformation assay is coupled with a nude mouse study. The combined system has the advantages of an *in vitro* short-term test, while enabling the study of mechanistic and/or sequential processes that occur during carcinogenesis. Although Dunkel *et al*. [10] described that $BeSO_4$-induced BALB/c-3T3 cell transformation, the tumorigenic potential of morphologically transformed cells *in vivo* has not been reported. The results, as shown in our study, indicate that transformed cells induced by $BeSO_4$ have a strong potential for tumorigenesis *in vivo* because transformed cells induced tumors rapidly in nude mice after inoculation.

It has been concluded by IARC that Be is potentially carcinogenic to humans and causes high incidence of tumors in animals, but the mechanism of carcinogenesis is not clearly understood. In mammalian cell systems, the major Be-induced damages reported in the literature are SCEs, CAs and cell transformation [9, 11, 12]. Activation of the K-*ras* protooncogene and inactivation of the *p53* tumor suppressor gene are events common to many types of human cancers. No K-*ras* gene mutations or less than 10% K-*ras* codon 12 GGT-GTT transversion, however, were detected in rat lung tumors induced by Be [29–31]. In our study, no mutations in K-*ras*

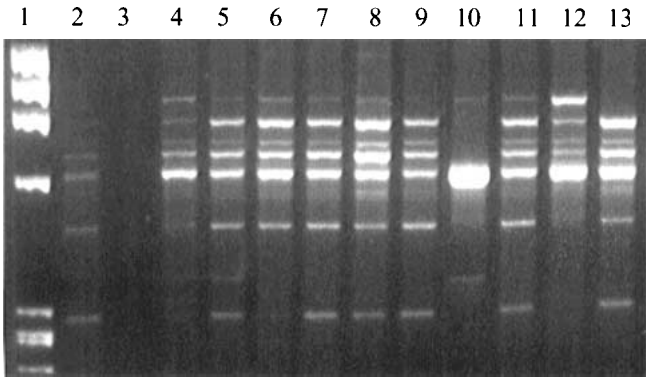

Fig. 3. Random amplified polymorphic DNA analysis of non-transformed and $BeSO_4$-transformed cell lines. Lane 1: Φ174/*Hae III* molecular weight marker; lane 2: non-transformed BALB/c-3T3 cells; lane 3: a blank; lanes 4–13: $BeSO_4$-transformed cells.

codon 12, 13 or 61 were found in any of the transformed cell lines. Therefore, these results suggest that K-*ras* activation is a rare event in the Be carcinogenic process. No *p53* gene mutations were observed in previous studies of animal lung tumors [28, 29]. It seems, therefore, that point mutations of these genes might not play a critical role in Be-induced animal lung tumors. Further studies were performed in our laboratory to determine whether alterations in certain proto-oncogenes and tumor suppressor genes were involved in Be-induced cell transformation and/or tumor development in nude mice. Both K-*ras* and c-*jun* were amplified in most transformed cell lines. We have previously observed c-*jun* overexpression by Western blotting in silica and glass fiber-transformed BALB/c-3T3 cells [32], however this result was not seen in the Be-transformed cells. The level of gene amplification of both K-*ras* and c-*jun* in the present study was relatively low (only 2 times the normal copy number). Therefore, it is possible that the level of increased expression was outside the lower limits of detection by Western blotting, yet still significant enough to influence cell transformation. Also, genomic alterations as observed by RAPD showed that some transformed cell lines were altered. Therefore, it is possible that cell transformation induced by $BeSO_4$ may be attributed, in part, to the gene amplification of K-*ras* and c-*jun* and that some $BeSO_4$-induced transformed cells possess neoplastic potential resulting from genomic instability.

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