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Gene Expression Profile in BALB/c-3T3 Cells Transformed With Beryllium Sulfate

Pius Joseph,* Timothy Muchnok, and Tong-man Ong

Molecular Epidemiology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia

Differential gene expression was studied to understand the potential molecular mechanism responsible for cell transformation and tumorigenesis induced by beryllium. Cell lines were derived from tumors developed in nude mice injected subcutaneously with BALB/c-3T3 cells morphologically transformed with beryllium sulfate. Using the Atlas mouse 1.2 cDNA expression microarray, the expression profiles of 1176 genes, belonging to several different functional categories, were studied in the tumor cells as well as in the nontransformed control cells. Expression of 18 genes belonging to two functional groups was found to be consistently and reproducibly different (at least twofold) in the tumor cells compared with the control cells. The functional groups and the differentially expressed genes are as follows: The cancer-related genes (nine genes) were the *ets*-related transcription factor activated by *ras,* colony-stimulating factor, A-*myb, sky, cot1,* c-*fos,* c-*jun,* c-*myc,* and R-*ras* proto-oncogenes. The DNA synthesis, repair, and recombination genes (nine genes) were the DNA replication licensing factor *MCM4*, the DNA replication licensing factor *MCM5*, the DNA mismatch repair gene *PMS2*, the DNA excision repair gene, the DNA mismatch repair gene *MSH2*, the ultraviolet excision repair gene *Rad23*, DNA ligase 1, *Rad51,* and *Rad52.* The differential gene expression profile was confirmed with reverse transcription–polymerase chain reaction using primers specific for the differentially expressed genes. In general, expression of the cancer-related genes was upregulated, while expression of genes involved in DNA synthesis, repair, and recombination was downregulated in the tumor cells compared with the control cells. Using c-*fos* and c-*jun,* two of the differentially expressed genes, as model genes, we have found that in the nontransformed BALB/c-3T3 cells, the beryllium-induced transcriptional activation of these genes was dependent on pathways of protein kinase C and mitogen-activated protein kinase and independent of reactive oxygen species. These results indicate that beryllium-induced cell transformation and tumorigenesis are accompanied by and are possibly a product of alterations in expression of genes related to cancer and to DNA synthesis, repair, and recombination.

Key words: microarray; cell transformation; tumorigenesis; mechanisms

INTRODUCTION

Metals, including beryllium (Be), are used extensively in industry. Further, significant amounts of Be have been detected in the environment as well as in food and water, and, therefore, there is a potential for extensive human exposure [1,2]. Exposure to Be induces a wide range of toxic responses, including chronic Be disease, acute pneumonitis, and chronic granulomatous lung disease [3].

There is strong experimental evidence that animals' exposure to Be results in an increase in the incidence of tumors [4]. Based on review of cohort studies by their working group, the International Agency for Research on Cancer has found a higher risk of lung cancer among workers occupationally exposed to Be compounds [5,6], and there are epidemiological data supporting excess cancer risk in humans exposed to Be compounds [7]. Although there is ample experimental and epidemiological evidence of the carcinogenic potential of Be, the underlying molecular mechanisms responsible for Be-induced carcinogenesis are not clearly understood.

Recent developments in genome research have shown the potential of studies investigating differential gene expression profiles to elucidate the cellular/molecular mechanisms responsible for chemical carcinogenesis. Therefore, using nontransformed (control) BALB/c-3T3 cells and cells developed from tumors grown in nude mice subcutaneously injected with BALB/c-3T3 cells that were morphologically transformed with beryllium sulfate ($BeSO_4$), we investigated differential gene expression to understand the possible mechanisms of Be-induced cell transformation and tumorigenesis.

*Correspondence to: MS 3014, Toxicology and Molecular Biology Branch, CDC/NIOSH, 1095 Willowdale Road, Morgantown, WV 26505.

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Abbreviations: RT, reverse transcription; PCR, polymerase chain reaction; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; ROS, reactive oxygen species; SOD, superoxide dismutase; PBS, phosphate-buffered saline.

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Furthermore, we elucidated some of the cellular mechanisms responsible for the Be-induced deregulation of two important proto-oncogenes, c-*fos* and c-*jun,* that were overexpressed during $BeSO_4$-induced cell transformation and subsequent tumorigenesis.

MATERIALS AND METHODS

Transformation of BALB/c-3T3 Cells and Development of Tumor Cell Lines

Early passages of mycoplasma-free BALB/c-3T3 cells (American Type Culture Collection, Rockville, MD) exhibiting contact inhibition were treated with 50–200 μg/mL $BeSO_4$ (Sigma Chemical Co., St Louis, MO) for 72 h, and the transformed foci were isolated after 3–5 wk. Approximately 10^6 cells derived from the foci were injected subcutaneously into nude mice to cause tumor development. Cell lines developed from the tumors were grown in minimum essential medium (Sigma Chemical Co.) containing 7.5% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Morphologic transformation of BALB/c-3T3 cells by $BeSO_4$ and the development of tumor cell lines were done previously in our laboratory, and the results have been published elsewhere [8].

Gene Expression Studies Using Mouse cDNA Expression Microarray

The gene expression profiles of the tumor cells and the nontransformed (control) cells were studied using the Atlas mouse 1.2 cDNA expression array (Clontech Laboratories, Palo Alto, CA), consisting of cDNAs for 1176 genes. The complete listing of the genes and their localization on the array are available from the manufacturer. One control and three tumor cell lines were used in the study. RNA isolated from the individual cell lines was analyzed for differential gene expression using the microarray. Total RNA, free of genomic DNA, nucleases, and other impurities, was isolated from the cells using the Atlas pure total RNA labeling system (Clontech Laboratories) according to the manufacturer's protocol. The quality and integrity of the isolated RNA were analyzed by ultraviolet spectrophotometry and by agarose gel electrophoresis, respectively [9].

Synthesis of cDNA was done with Moloney murine leukemia virus reverse transcriptase in the presence of gene-specific primers (Clontech Laboratories) and [^{32}P]dATP, according to the manufacturer's protocol. Labeled cDNA probes were purified on ChromaSpin-200 columns (Clontech Laboratories), and fractions corresponding to cDNA were pooled and used in the hybridization. Typically, 15×10^6 cpm of ^{32}P-labeled cDNA probes was used per membrane. Prehybridization and hybridization of the cDNA expression arrays were done at 68°C for 1 h and 16 h, respectively. Images were scanned and quantitated with phosphorimaging, and data analysis was done with the AtlasInfo database (Clontech Laboratories).

Validation of Gene Expression Profile Study Results

A total of 18 genes that were differentially expressed in the tumor cells compared with the nontransformed cells, based on the results of microarray analysis, were used in this experiment. These genes belong to the functional categories of cancer-related genes (nine genes) and DNA synthesis, damage repair, and recombination genes (nine genes). A listing of all differentially expressed genes is provided in Table 1. Each of these genes, with the exception of genes encoding MAX protein, Lupus Ku autoantigen protein p86, and the ATP-dependent DNA helicase II subunit, was found to show at least a twofold difference in expression in the tumor cells compared with the control, nontransformed cells. Moloney murine leukemia virus reverse transcriptase–catalyzed synthesis of cDNAs from RNA was done using the Advantage RT-for-PCR kit (Clontech Laboratories), according to the procedure provided by the manufacturer.

The sequence of the gene-specific primers, with the exception of c-*fos,* c-*jun,* c-*myc,* and β-*actin,* used to amplify the differentially expressed genes are the proprietary information of Clontech Laboratories and are available from the manufacturer. Information about the primers used to amplify c-*fos,* c-*jun,* c-*myc,* and β-*actin* genes have been presented previously [10]. All the reagents used for polymerase chain reaction (PCR) amplification were purchased from Promega Corporation (Madison, WI). The PCR reaction mixture consisted of cDNA equivalent to 125 ng of RNA, 200 μM dNTPs, 1.25 mM $MgCl_2$, 50 pmol each of the gene-specific primers, and 1.25 U of Taq polymerase in a total volume of 50 μL. The PCR amplification procedure consisted of 25 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 2 min, followed by a single extension of 7 min at 72°C. The 25 cycles were within the linear range of PCR amplification (data not provided).

To minimize variations due to experimental conditions, amplification of β-actin (housekeeping gene) and the target genes was done simultaneously. Moreoever, all the PCR reagents, with the exception of the cDNA template, were mixed together to prepare the master mix, and the same master mix was used for amplification of the housekeeping gene and the target genes of the control and the tumor cells. The PCR products were resolved by 1% agarose gel electrophoresis, and the ethidium bromide–stained images were captured using the Eagle Eye II (Stratagene, La Jolla, CA) gel documentation system and analyzed using National

Table 1. Gene Expression Profile in Cells Developed from Tumors Grown in Nude Mice Injected Subcutaneously with BALB/c-3T3 Cells Morphologically Transformed with Beryllium Sulfate

Gene*	Coordinate†	Expression (fold change)‡
Cancer-related genes		
net, ets-related protein activated by *ras*	CO4a	+4.8
A-*myb* proto-oncogene	CO4m	+4.1
Colony-stimulating factor	CO5n	+2.6
sky proto-oncogene	CO6g	+4.1
*cot*1 proto-oncogene	CO7l	+2.2
c-*fos*	CO4c	+3.8
c-*jun*	CO4d	+4.2
c-*myc*	CO5c	+3.7
R-*ras*	CO8b	+4.6
DNA synthesis, repair, and recombination genes		
MCM4 DNA replication licensing factor	F10f	−7.3
MCM5 DNA replication licensing factor	F10g	−3.2
PMS2 DNA mismatch repair protein	F101	−Infinite§
DNA excision repair protein	F11a	−3.1
MSH2 DNA mismatch repair protein	F11e	−6.6
Rad23 UV excision repair protein homolog	F11g	−11.5
DNA ligase 1	F12f	−7.4
Yeast DNA repair protein *Rad52* homolog	F12h	−4.4
Yeast DNA repair protein *Rad51* homolog	F12n	−5.8

*Only genes whose differential expression was confirmed by RT-PCR are listed. The differential expression of genes encoding MAX protein, Lupus Ku autoantigen protein p86, and the ATP-dependent DNA helicase II 86-kDa subunit could not be confirmed with RT-PCR.
†Position on the microarray.
‡Positive and negative numbers represent upregulated and downregulated expression levels in the tumor cells compared with the control cells. The numbers represent results from analysis of the microarray. RT-PCR analysis, in general, showed similar results, and these results are not presented.
§Expression level in the tumor cells was undetectable.

Institutes of Health Image Analysis software (NIH, Bethesda, MD).

$BeSO_4$-Induced Overexpression of c-*fos* and c-*jun* Proto-oncogenes

As a prelude to elucidating the cellular/molecular mechanisms underlying $BeSO_4$-induced deregulation of gene expression, the potential of $BeSO_4$ to induce the expression of the proto-oncogenes c-*fos* and c-*jun* was investigated. Nontransformed BALB/c-3T3 cells growing at 60–70% confluence were serum-starved for 24 h and exposed to $BeSO_4$ at concentrations ranging from 50 to 1000 μg/mL medium for 2 h. (The time required for optimum induction of expression of these genes by $BeSO_4$ was obtained from a separate experiment.)

Mechanisms of $BeSO_4$-Induced Overexpression of c-*fos* and c-*jun* Proto-oncogenes

To determine the molecular mechanisms responsible for the $BeSO_4$-induced deregulation of gene expression, the expression of c-*fos* and c-*jun* was studied in the nontransformed BALB/c-3T3 cells treated with $BeSO_4$. The involvement of hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), transcription, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) was investigated using the following specific inhibitors/scavengers: catalase (for H_2O_2), superoxide dismutase (SOD; for O_2^-), actinomycin D (for transcription), RO-31-8220 (for PKC), and PD 98059 (for MAPK). The experiments were done as follows: Nontransformed BALB/c-3T3 cells exponentially growing in 75-cm^2 flasks at 60–70% confluence were serum-starved for 24 h. Subsequently, the cells were treated for 1 h at 37°C with the various modulators (inhibitors/scavengers) at the following final concentrations: catalase (1000 U/mL), SOD (1000 U/mL), actinomycin D (5 mg/mL), RO-31-8220 (5 μM), and PD 98059 (100 μM).

The cells were washed with phosphate-buffered saline (PBS) and treated with $BeSO_4$ at a final concentration of 400 μg/mL of the cell culture medium for 2 h. (The dose and duration of exposure to $BeSO_4$ resulting in the optimum induction of genes were determined in separate experiments.) At the end of the exposure period, the cells were rinsed with PBS and allowed to grow in fresh growth medium for 1 h, and RNA was isolated from the cells to determine the expression level of c-*fos* and c-*jun* by Northern blotting. Twenty micrograms of total RNA from each sample was denatured and separated

by electrophoresis on an agarose-formaldehyde gel and transferred to positively charged nylon membranes (Roche Molecular Biochemicals, Indianapolis, IN) following standard procedures [9]. PCR-amplified c-*fos* and c-*jun* cDNAs were labeled with digoxigenin (Roche Molecular Biochemicals) by random prime labeling and were used as the probes for hybridization. The hybridized target genes were detected using the Dig-Easy Detection System (Roche Molecular Biochemicals), according to the procedure provided by the manufacturer.

RESULTS

Gene Expression Profile

Even though the Atlas mouse 1.2 cDNA expression microarray consists of cDNAs for 1176 genes belonging to several functional groups and arranged into six quadrants, we found significant and consistent differential expression of genes belonging to only two functional groups: cancer-related genes and DNA synthesis, repair, and recombination genes, clustered in quadrants C and F, respectively, of the microarray. A typical image of quadrants C and F of the hybridized microarray is presented in Figure 1. The result, which is an average of three hybridization experiments, is summarized in Table 1. Only those genes exhibiting at least a twofold difference in expression in the tumor cells compared with the control cells were considered significant and are presented in Table 1. Expression of all the genes was normalized by comparing the expression of several housekeeping genes in the control and in the tumor cells.

It is noteworthy that in the microarray, several of the genes present in the same quadrants as the cancer-related genes and DNA repair genes did not show differences in their expression between the control and the tumor cells. Among the cancer-related genes, the highest change in expression was noticed in the case of *Net,* ets-related transcription factor activated by *ras* (+4.8-fold), whereas the lowest alteration was noticed in the case of the *cot1* proto-oncogene (+2.2-fold). Unlike the cancer-related genes, expression of genes related to DNA synthesis, repair, and recombination was lower in the tumor cells than in the nontransformed cells, and the fold difference in expression was, in general, higher than those of the cancer-related genes. The highest difference was noticed in the case of the DNA mismatch repair gene *PMS2*, expression of which was undetectable in the tumor cells.

Validation of Gene Expression Profile

The results of the reverse transcription (RT)–PCR experiments confirmed the gene expression profile results obtained with the microarray, with the exception of the genes encoding MAX protein, Lupus Ku autoantigen protein P86, and the ATP-dependent DNA helicase II 86 KDa subunit. These results are presented in Figure 2A and B. The housekeeping gene β-actin, used for normalization of the expression profile, did not show any change in expression in the cells, whereas the expression of all other genes studied was different in the tumor cells compared with the control cells. Similar results were obtained with the $BeSO_4$-transformed BALB/c-3T3 cells that subsequently were injected into the nude mice to induce tumors from which the cell lines used in this investigation were developed (data not presented).

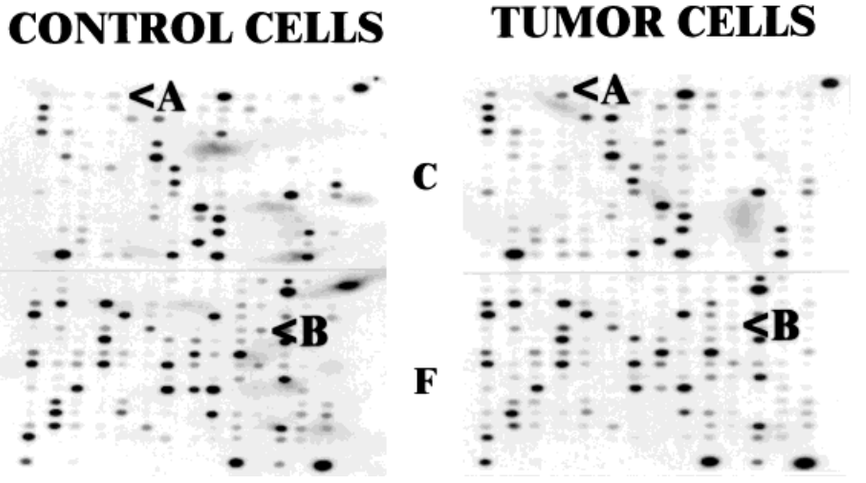

Figure 1. Gene expression profile in the control and tumor cells, as analyzed by cDNA expression microarray. ^{32}P-labeled cDNA probes were synthesized by reverse-transcribing RNA from control and tumor cells and were hybridized to the Atlas mouse 1.2 cDNA expression array, as described in Materials and Methods. The phosphorimage of a representative hybridization experiment is presented. Only quadrants C (cancer-related genes) and F (DNA synthesis, repair, and recombination genes) of the microarray are shown. The arrows indicate *net*, *ets*-related protein activated by ras (A) and the DNA mismatch repair gene *MSH2* (B).

Figure 2. Confirmation of cDNA array results by RT-PCR. Total RNA isolated from the control and the tumor cells was reverse-transcribed as described in Materials and Methods. The cancer-related genes (A) and the cDNA synthesis, repair, and recombination genes (B) were PCR-amplified using primers specific for the respective genes. The PCR products were resolved by agarose gel electrophoresis, and the result of a representative experiment is presented. C, control (nontransformed cells); T, tumor cells.

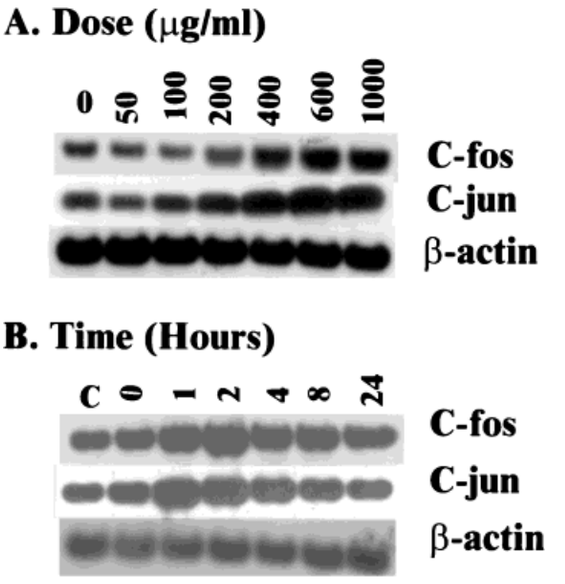

Figure 3. Induction of overexpression of c-*fos* and c-*jun* proto-oncogenes in BALB/c-3T3 cells treated with $BeSO_4$. (A) Dose (micrograms per milliliter): Nontransformed BALB/c-3T3 cells were serum-starved for 24 h and treated with $BeSO_4$ at doses ranging from 0 to 1000 μg/mL medium for 2 h. The cells were rinsed in PBS and allowed to grow in control medium for 2 h. RNA isolated from the cells was analyzed by Northern hybridization to determine the expression of c-*fos,* c-*jun,* and β-actin genes. The results shown are representative of three independent experiments. (B) Time (hours): Cells were exposed to 400 μg of $BeSO_4$/mL medium for 2 h. Subsequently, the cells were rinsed with PBS and allowed to grow in fresh medium for 24 h. At the indicated time intervals, RNA was isolated, and expression of c-*fos,* c-*jun,* and β-actin was determined by Northern hybridization. The results shown are representative of three independent experiments.

$BeSO_4$-Induced Overexpression of c-*fos* and c-*jun* Proto-oncogenes

Exposure of the nontransformed BALB/c-3T3 cells to a single dose of $BeSO_4$ resulted in overexpression of both the c-*fos* and c-*jun* proto-oncogenes. The effect was dose dependent, and a significant induction of expression was noticed at doses of $BeSO_4$ ranging from 200 μg/mL to 1000 μg/mL of the growth medium (Figure 3A). $BeSO_4$-induced overexpression of the proto-oncogenes was also time-dependent (Figure 3B). The transient induction of expression of the genes was highest at 2–4 h after the termination of the 2-h exposure to $BeSO_4$; by the end of 24 h, the expression of the genes had returned almost to basal levels.

Effect of Modulators on $BeSO_4$-Induced Overexpression of c-*fos* and c-*jun* Proto-oncogenes

Exposure of the nontransformed BALB/c-3T3 cells to a single dose of $BeSO_4$ (400 μg/mL for 2 h) resulted in significant overexpression of the c-*fos* and c-*jun* proto-oncogenes. Pretreating the cells with the various modulators for 1 h immediately before their exposure to $BeSO_4$ resulted in varying degrees of inhibition of $BeSO_4$-induced overexpression of c-*fos* and c-*jun* proto-oncogenes. In general, the effect of the various modulators was similar for both the proto-oncogenes (Figure 4). Pretreating the cells with SOD and catalase, which are scavengers of reactive oxygen species (ROS; O_2^- and H_2O_2, respectively), had no significant effect on the expression of the proto-oncogenes. Actinomycin D, RO-31-8220, and PD 98059, inhibitors specific for transcription, PKC, and MAPK, respectively, resulted in significant inhibition of $BeSO_4$-induced expression of the proto-oncogenes (Figure 4).

DISCUSSION

The development of cancer is the result of a series of molecular changes taking place in the cell. These events may lead to changes in the expression of numerous genes conferring proliferative, invasive, or metastatic potential on normal cells, resulting in the development of the malignant phenotype. Cell transformation, coupled with the tumorigenesis assay using immune-deficient mice, exhibits characteristics analogous to in vivo carcinogenesis and has long been used as a surrogate to study the carcinogenic potential of chemicals [11]. Because of the close similarities between cell transformation in vitro and multistage transformation in vivo, cell transformation assays with BALB/c-3T3 cells also

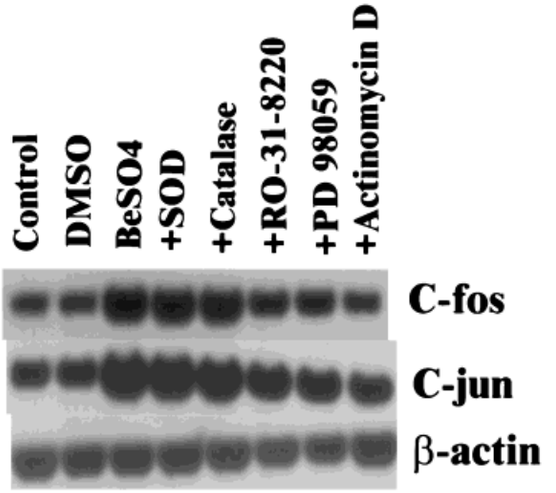

Figure 4. Mechanisms of $BeSO_4$-induced overexpression of c-*fos* and c-*jun* in BALB/c-3T3 cells. Nontransformed BALB/c-3T3 cells were serum-starved for 24 h and treated with the various modulators for 1 h at the final concentrations described in Materials and Methods. The cells were rinsed with PBS and treated with 400 μg of $BeSO_4$/mL medium for 2 h. Two hours after the termination of exposure to $BeSO_4$, RNA was isolated from the cells, and expression of c-*fos,* c-*jun,* and β-actin was determined by Northern hybridization. The results shown are representative of three independent experiments.

have been employed as a predictive tool for assessing the potential for chemical carcinogenesis as well as to study the cellular mechanisms of chemical carcinogenesis [12].

The proto-oncogenes c-*fos,* c-*jun,* and c-*myc,* which are overexpressed in Be-induced tumor cells, are all members of the immediate early-response genes [13]. These genes are overexpressed in several other tumor samples as well [10,14]. Both c-*fos* and c-*jun* are members of the activator protein-1 complex, and they encode a transcription factor capable of deregulating the expression of several target genes, resulting in the increased proliferation and morphologic transformation of cells [15]. The *cot1* proto-oncogene encodes for a MAPK kinase kinase that activates the mitogenic signaling cascades through phosphorylation of target genes [16]. Expression of the *cot1* gene has an important role in epithelial cell transformation and tumor promotion [17], and its overexpression has been reported in tumor tissues [18]. The *myb* family of genes includes several proto-oncogenes and encodes transcription factors involved in cell proliferation and differentiation [19], and the oncogenic activation of these genes leads to cell transformation [20]. The involvement of the *ras* family of oncogenes in carcinogenesis has been very well established by studies involving animal and human tumor samples [21]. Oncogenic signals originating from the *ras* genes are mediated in part by transcription factors that belong to the *ets* gene family [22]. The overexpression of both *ras*-related and *ets*-related genes in $BeSO_4$-induced tumor cells suggests that *ras*-mediated oncogenic signals also are involved in $BeSO_4$-induced cell transformation and tumorigenesis.

DNA synthesis, repair, and recombination are essential cellular processes required for normal development, growth, and functioning of cells. In general, the genes regulating DNA synthesis, repair, and recombination serve to maintain the fidelity of DNA replication. Lack or loss of expression of these genes has been found to be a factor predisposing to the development of diseases, most notably, cancer [23]. The relationship between defects in DNA repair genes and development of tumorigenesis is best illustrated in the case of hereditary nonpolyposis colon cancer. Defects in such DNA repair genes as *RAD51* [24], *hMSH2* [25], *hMLH1* [26], and *PMS2* [27] have been shown to perturb genomic integrity and eventually lead to hereditary nonpolyposis colon cancer. Furthermore, several members of the DNA repair gene family can interact with cancer-related genes such as *BRCA1, BRCA2,* and *p53,* which are key determinants in the susceptibility of cells to malignant transformation [28]. DNA ligase 1, whose expression was significantly lower in the $BeSO_4$-induced tumor cells, mediates essential functions in mammalian cells [29]. Cells with decreased DNA ligase 1 activity show an extraordinarily high rate of mutation and chromosome loss and an elevated chromatid-exchange frequency. They also show broad sensitivity to DNA-damaging agents, suggesting a generalized deficiency in DNA repair [30]. Deficiency in DNA-repair capacity is an established predisposing factor in tumorigenesis [31]. With respect to its capacity to inhibit the DNA-repair process, Be has a marked similarity to cadmium [31].

Even though detailed study of the signal transduction cascades that might have been involved in Be-induced differential gene expression was not conducted in the present investigation, it appears that the cell proliferation signal initiated by $BeSO_4$ was transduced through MAPK and PKC. This conclusion is based on the observation that PD 98059 and RO-31-8220, inhibitors of MAPK [32] and PKC [33], respectively, were able to interfere with Be-induced overexpression of the proto-oncogenes c-*fos* and c-*jun.* Similar inhibitory effects of these agents have been reported previously with respect to the induction of overexpression of these proto-oncogenes by another metal, cadmium [10,34]. Even though the mitogenic signal cascades induced by $BeSO_4$ are not understood fully, based on our results and those of others [10,34], it may be possible to conclude that the mitogenic signals induced by metals in general converge at MAPK and PKC, which phosphorylate signaling proteins to stimulate the activation of proto-oncogenes controlling cell proliferation. Furthermore, the overexpression of c-*fos* and c-*jun* in response to $BeSO_4$ exposure appears to be the result of transcriptional activation of genes,

as evidenced by the lack of any significant induction of these genes in the cells pretreated with the inhibitor for transcription, actinomycin D.

Acting directly on cells or indirectly through stimulation of phagocytes, metals can induce the production of ROS, which in turn are implicated in many aspects of metal-induced tumorigenesis [35,36]. In fact, generation of ROS and associated oxidative stress have been found to be responsible for carcinogenesis after exposure to such metals as chromium, arsenic, cadmium, nickel, and mercury [35]. Based on our data with the scavengers of ROS (SOD and catalase), it does not appear that generation of ROS and the associated oxidative stress were involved in Be-induced cell transformation and tumorigenesis. This conclusion derives from the observation that Be-induced overexpression of c-*fos* and c-*jun* was unaffected by pretreatment of the cells with the scavengers of ROS. Both c-*fos* and c-*jun* are members of the immediate early-response gene family [23] and are known to respond to ROS [37]. Moreover, exposure of cells to SOD and catalase, as in the present study, is known to modulate the cellular level of ROS, which in turn influences the expression of c-*fos* and c-*jun* [22]. Results of the in vitro experiments presented here, however, do not rule out the possibility of the involvement of ROS associated with inflammatory responses in Be-induced carcinogenesis in vivo, where generation of ROS by inflammatory cells may play a critical role.

The results of the present study show that gene-profiling experiments employing cDNA expression arrays are highly useful in the identification of differential gene expression patterns during cell transformation and tumorigenesis. Identification of the differentially expressed genes, in turn, is helpful in predicting as well as understanding the potential cellular/molecular mechanisms of chemical carcinogenesis. Thus, based on the results presented in this study, it appears that Be-induced downregulation of the expression of genes involved in DNA synthesis, repair, and recombination, in conjunction with upregulation of cancer-related genes, may be responsible for conferring the proliferative advantage resulting in cell transformation and tumorigenesis. While the present findings elucidate some of the potential mechanisms of Be-induced cell transformation and tumorigenesis, further in vivo studies and evidence of the changes in cellular levels of the proteins encoded by the suggested genes are required to confirm the biological significance of the results of the present study.

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