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Transcription Profiles of Human Cells in Response to Sodium Arsenite Exposure

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ABSTRACT *: Arsenic exposure is associated with several human diseases, including cancers, atherosclerosis, hypertension, and cerebrovascular diseases. In cultured cells, arsenite, an inorganic arsenic compound, was demonstrated to interfere with many physiological functions, such as enhancement of oxidative stress, delay of cell cycle progression, and induction of structural and numerical changes of chromosomes. The objective of this study is to investigate the effects of arsenic exposure on gene expression profiles by colorimetric cDNA microarray technique. HFW (normal human diploid skin fibroblasts), CL3 (human lung adenocarcinoma cell line), and HaCaT (immortalized human keratinocyte cell line) were treated with 5 μM or 10 μM sodium arsenite for 6 or 16 h, respectively. By a dual-color detection system, the expression profile of arsenite-treated cultures was compared to that of control cultures. Several genes expressed differentially were identified on the microarray membranes. For example, MDM2, SWI/SNF, ubiquitin specific protease 4, MAP3K11, RecQ protein-like 5, and Ribosomal protein L10a were consistently induced in all three cell types by arsenite, whereas prohibitin, cyclin D1, nucleolar protein 1, PCNA, Nm23, and immediate early protein (ETR101) were apparently inhibited. The present results suggest that arsenite insults altered the expression of several genes participating in cellular responses to DNA damage, stress, transcription, and cell cycle arrest.*

Key Words *: Arsenite, Expression profile, cDNA microarray, Human cells*

I. INTRODUCTION

Arsenic carcinogenicity was evidenced in numerous epidemiological studies during the past several decades (Abernathy *et al.*, 1999; IARC, 1987). The peripheral lymphocytes of human population with chronic arsenic exposure manifested elevated frequencies on sister chromatid exchanges, chromosome aberrations, and micronuclei (Jha *et al.*, 1992; Lerda, 1994; Gonsebatt *et al.*, 1997). In Blackfoot endemic area, the residents with increased chromosome aberration frequency in peripheral lymphocytes were associated with increased risks of cancer development (Liou *et al.*, 1999). Cultured cells treated with inorganic arsenic also revealed with increased frequencies on sister chromatid exchanges, chromosome aberrations, micronuclei, and morphological cell transformation (Jha *et al.*, 1992; Lee *et al.*, 1985; Lee *et al.*, 1985; Oya *et al.*, 1996; Yih and Lee, 1999). Numerous studies have also shown that arsenic treatment resulted in cell cycle arrest (Lee *et al.*, 1989; Yih *et al.*, 1997), mitosis disturbance (Yih *et al.*, 1997; Huang and Lee, 1998), aneuploidy (Yih *et al.*, 1997), and apoptosis (Wang *et al.*, 1996; Curr *et al.*, 1999) in a variety of human cell lines. These studies indicated that arsenite exposure resulted in pleiotropic responses to arsenite insults in 3 human cell types.

In response to injury and stress, a variety of cellular defense and repair pathways should be activated to maintain the integrity of cells or eliminate the seriously damaged cells. A series of early responses to ionic radiation (IR) including activation of p53 and NFkB, induction of protein re-localization, engagement of signal cascades, and transcriptional induction of *cip1* and *MDM2* were demonstrated in a variety of cells (Little, 2000; Schmidt *et al.*, 2000; Rotman and Shiloh, 1999). The cellular responses to the insults or stresses were complicated but important for determining the fate of cells against the encountered adverse effect (Amundson *et al.*, 1999). To investigate the transcriptional stress-responses by monitoring the expression profile of a panel of genes

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Abbreviations: AU, arbitrary unit

may help unveil the role of genes involved in regulating cellular responses to a specific stress and elucidate the underlying mechanism(s) involved in stress-induced effect (Nuwaysir *et al.*, 1999).

The cDNA microarray is an efficient tool for solving the difficulties in simultaneously analyzing the expression levels of a large number of genes (Afshari *et al.*, 1999; Bartosiewicz *et al.*, 2000; Harrington *et al.*, 2000; Watson *et al.*, 1998). In this study, profiling expression patterns in 3 arsenite-exposed human cell types, including foreskin fibroblasts (HFW), lung adenomcarcinoma cell line (CL3), and immortal keratinocytes (HaCaT), were examined by the cDNA microarray colorimetric detection system (Chen *et al.*, 1998). The present results showed that arsenite apparently induced the expression of a set of genes associated with DNA damage, stress, apoptosis, and cell cycle arrest in these 3 cell types. These results indicated that arsenite might trigger a series of signaling cascade responsible for arsenite-induced insults or stress.

II. MATERIALS AND METHODS

1. Cell culture

HFW cells derived from newborn foreskin and CL3 cells derived from a human lung adenocarcinoma were kindly obtained by Dr. W. N. Wen (National Taiwan University, Taipei) and Dr. P. C. Yang (National Taiwan University Hospital, Taipei), respectively. HaCaT cells, spontaneously immortalized human keratinocytes, were kindly provided by Dr. N. E. Fusenig (German Cancer Research Center, Heidelberg, Germany). HFW and HaCaT cells were routinely maintained in Dulbecco's modified Eagle medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 0.37% sodium bicarbonate, and antibiotics, and cultured at 37°C in an incubator with humidity-saturated air and 10% CO_2 (Lee *et al.*, 1989). CL3 cells were grown in F12 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum, 0.22% sodium bicarbonate, and antibiotics, and incubated at 37°C in an incubator with humidity-saturated air and 5% CO_2 (Lee and Ho, 1994).

2. Arsenite treatment

Sodium arsenite (Merck, Darmstadt, Germany), an inorganic trivalent arsenic, was freshly prepared by dissolving in distilled water before experimental manipulation. The logarithmically growing cells were treated with sodium arsenite at 5 or 10 μM for 6 to 24 h, respectively. Cytotoxicity to arsenite was determined by the colony forming assay as previously described (Yih *et al.*, 1997).

3. mRNA and cDNA probe preparation and Southern hybridization

At the end of arsenite treatment, total cellular RNA was extracted with the Tri-reagent (Molecular Research Center, Inc., Ohio), and mRNA was subsequently isolated with Oligotex-dT resin (Qiagen, Germany). Biotin- or digoxigenin-labeled cDNA probes were prepared by reverse transcription. In brief, 1 μg isolated mRNA was reversely transcribed to cDNA with 200 units of MMLV reverse transcriptase in a 50 μl solution containing 6 μM random primers (GIBCO), 0.5 mM each dATP, dCTP, dGTP, 40 μM dTTP, 40 μM biotin-16-dUTP or 40 μM digoxigenin-11-dUTP (Boehringer Mannheim, Germany), 10 mM dithiothreitol, and 0.5 unit/ml RNase inhibitor (GIBCO). The reaction mixture was first incubated at 25°C for 10 min and then switched to 42°C for 90 min. Afterward, the reaction was terminated by heating at 99°C for 5 min. The remaining RNA was digested by addition of 5.5 μl of 3 N NaOH and incubation at 55°C for 30 min. After neutralization by addition of 5.5 μl of 3 N acetic acid, the cDNA probes were precipitated by addition of 50 μl 7.5 M ammonia acetate, 20 μg linear polyacrylamide as carrier, 375 μl absolute ethanol, and water to make a total of 525 μl.

The membrane carrying double-stranded cDNA targets covered 568 human ESTs and 8 plant genes. Two cDNA probes, one from untreated control and labeled with biotin-16-dUTP and the other from arsenite-treated cells and labeled with digoxigenin-11-dUTP, were mixed in equal amounts and hybridized to the prehybridized membrane at 65°C for 16 h as previously reported (Chen *et al.*, 1998). The membrane was then thoroughly washed for 2 times with 2× SSC containing 0.1% sodium dodecyl sulfate for 5 min at

room temperature and followed by 3 washes with 0.1× SSC containing 0.1% sodium dodecyl sulfate at 65°C for 15 min each.

4. Colorimetry detection and image analysis

After thoroughly washing, the membrane was blocked in 1% blocking solution (Boehringer Mannheim, Germany) containing 2% dextran sulfate at room temperature for 1 h and then rinsed with 1× Tris-buffered saline (1×TBS, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl). The membranes were then incubated with β-galactosidase-conjugated streptavidin (1:700 dilution, GIBCO) and alkaline phosphatase-conjugated anti-digoxigenin antibody (1:10000 dilution, Boehringer Mannheim, Germany) for 1 h at room temperature. At the end of incubation, the membranes were washed 3 times with 1×TBS and then incubated with 1×TBS containing 1.2 mM X-gal, 1 mM $MgCl_2$, 3 mM $K_2Fe(CN)_6$, and 3 mM $K_4Fe(CN)_6$ at 37°C for 30 min till the blue color was well-developed. After briefly rinsed in distilled water, the membrane was further incubated with Fast Red TR/naphthol AS-MX substrate (Pierce, IL) for 30 min at room temperature for alkaline phosphatase reaction. The color development was terminated by addition of phosphate-buffered saline containing 20 mM disodium ethylenediaminetetraacetate. The colors of spots on membranes were scanned under a flatbed scanner at 3048 dots per inch and separated into artist's subtractive primaries (cyan, magenta, and yellow). The determination of gene expression levels was performed by a computer program written in-house (Chen *et al.*, 1998).

III. RESULTS AND DISCUSSION

1. Cytotoxicity of arsenite

By colony forming efficiency assay, the survival rates of HFW cells treated with 5 μM arsenite for 6 h and 16 h were 70% and 30%, respectively. The survival rates of CL3 cells treated with 5 and 10 μM arsenite for 6 h were 74% and 58%, respectively, while those of HaCaT cells were 80% and 50%, respectively. These results indicated that equivalent toxicity doses of arsenite to these 3 cell types were used in the following experiments.

2. Determination of differentially expressed genes by colorimetirc cDNA microarray

To determine the cut-off range for differentially expressed genes, cDNA obtained from the untreated HFW cells were separately labeled with biotin-16-dUTP or digoxigenin-11-dUTP. These labeled-cDNA

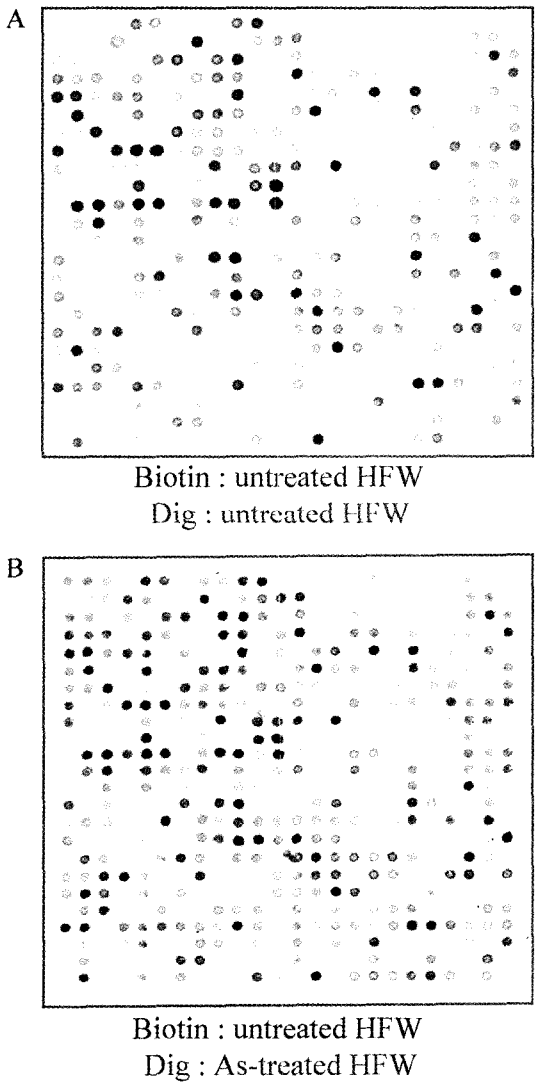

Fig. 1. Images of cDNA microarray membrane. HFW cells were untreated or treated with 5 μM arsenite for 6 h. The mRNA were extracted and reversely transcribed to cDNA in the presence of biotin-16-dUTP or digoxigenin-11-dUTP. The cDNA probes were hybridized with cDNA microarray membranes and colorimetrically detected as described in "Materails and Methods". (A) Membrane hybridized with probes that were mixed by an equal amount of bitoin- and Dig-labeled cDNA from untreated HFW cells. (B) Membrane hybridized with probes that were mixed with equal amounts of bitoin-labeled cDNA from untreated HFW and Dig-labeled cDNA from arsenite-treated HFW cells.

probes were then mixed in 1:1 ratio and hybridized to a 576-gene microarray membrane. After color development, the image shown in Fig. 1A was digitized by the aid of a computer. The scatter plot (cyan to magenta) of the intensity of each spot and the regression line (red) were shown in Fig. 2A. As expected, almost all spots (genes) were located within the 99% prediction interval (defined by 2 green lines). The vertical distance from each spot to the regression line is ranged from 6 (above the regression line or near cyan) to -3 (below the regression line or near magenta) arbitrary units (AU). Therefore, we defined a cut-off range between -3 to 6 AU for differential displayed genes. An example of a micrarray membrane hybridized with cDNA probes derived from the untreated HFW cells (labeled with biotin-16-dUTP) and arsenite-treated HFW cells (labeled with digoxigenin-11-dUTP) was shown in Fig. 1B. The scatter plot of cyan intensity to magenta of each spot was shown in Fig. 2B. The plot shown in Fig. 2B was obviously much dispersed than that in Fig. 2A. By placing the 99% prediction interval derived from Fig. 2A into Fig. 2B, many spots lied outside the 99% prediction interval, i.e. the vertical distance of these spots to the regression line was either larger than 6 AU or smaller than -3 AU. These spots were considered as genes with significant differential expression.

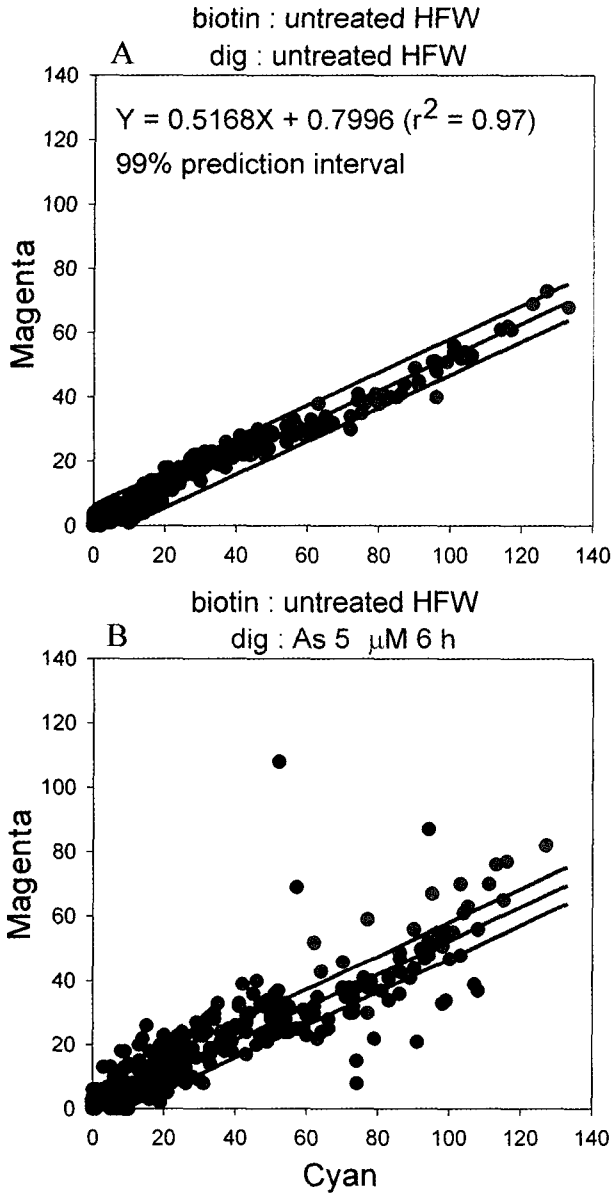

Fig. 2. Regression analysis on the color distribution of microarray gene spots. Scatter plots were data obtained from cyan and magenta value of each spot. (A) Image analysis data from the image of Fig. 1 (A). (B) Image analysis data from the image of Fig. 1 (B). The red line was the linear regression line obtained from scatter spots of Fig. 2 (A). The 2 green lines defined the edges of 99% prediction intervals.

3. Genes that are commonly up- or down-regulated by arsenite in 3 cell types

The use of cDNA microarray technique enables us to compare hundreds to thousands of genes simultaneously in a single hybridization experiment and to screen for differential gene expression. To understand the effects of arsenite on gene expression profiles, HFW, CL3 and HaCaT cells were treated with 5 or 10 μM arsenite for 6 or 16 h, respectively. As compared their expression profiles to the untreated control culture, 23 genes among the 568 human ESTs spotted on the microarray membrane were up regulated in all of these 3 cell types under different treatment conditions (Table 1). The products of these 23 genes were functionally involved in mitogenic signaling pathway (*MAP3K11*, *JAK1*), negative regulation of p53 (*MDM-2*), chromatin assembly (*SWI/SNF*), and transcription (*cyclin C*). The induction of *SWI/SNF* expression by arsenite in all cell types implies that arsenite may affect chromatin structure and hence alter gene expression.

In addition, RecQ protein-like 5, a DNA helicase involved in DNA replication, recombination, and repair, was also induced in all 3 cell types by arsenite indicated the deleterious effects of arsenite on genome integrity. *Ribosomal protein L10a*, homologous to heat

Table 1. The 23 genes induced by arsenite in 3 human cell types

Accession No.	Name in Unigene database	Symbol
R80779	mitogen-activated protein kinase kinase kinase 11	MAP3K11
R54687	CDC-like kinase 3	CLK3
W95001	cell division cycle 25C	CDC25C
AA164211	cyclin C	CCNC
N21348	menage a trois 1 (CAK assembly factor)	MNAT1
H13638	mouse double minute 2, human homolog of; p53-binding protein	MDM2
R80096	nuclear protein, ataxia-telangiectasia locus	NPAT
N91935	JAK binding protein	SSI-1
H60824	protein kinase C, theta	PRKCQ
W71984	tumor necrosis factor receptor superfamily, member 12	TNFRSF12
R63773	placental growth factor, vascular endothelial growth factor-related protein	PGF
R94403	apelin; peptide ligand for APJ receptor	APELIN
H18190	Janus kinase 1 (a protein tyrosine kinase)	JAK1
AA115793	CASP8 and FADD-like apoptosis regulator	CFLAR
AA033643	ubiquitin specific protease 4 (proto-oncogene)	USP4
T97648	sarcoma amplified sequence	SAS
R48588	HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 1	HRMT1L1
H30758	glucose phosphate isomerase	GPI
H45977	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCA2
R32075	RecQ protein-like 5	RECQL5
R23247	ribosomal protein L10a	RPL10A
H51797	EST	--
R22058	EST	--

shock protein 27, is involved in cellular resistance to oxidative stress (Mendez *et al.*, 2000). The results that arsenite significantly induced expression of ribosomal protein L10a gene in all three human cells were consistent to that arsenite could induce oxidative stress in a variety of cell systems (Wang *et al.*, 1996; Lee and Ho, 1995; Lynn *et al.*, 1998). Whether this gene could serve as a potentially good candidate for arsenic exposure marker warrants our further investigation. Unfortunately, the reports concerning the roles of genes shown in Table 1 in arsenite toxicity and carcinogenicity were limited.

Table 2 listed 10 consistently down-regulated genes by arsenite treatment in these 3 cell types. The gene products of *proliferating cell nuclear antigen*, *CDK 4*, *cyclin D1*, and *immediate early protein 1* were involved in cell cycle progression from G1 to S phase. These results confirmed the inhibitory effects of arsenite on cell cycle progression (Yih and Lee, 1999; Yih *et al.*, 1997; Huang and Lee, 1998).

4. The effects of arsenite on the expression profiles of detoxification-related genes

To understand how cells response to arsenite insults, we examined the expression profiles of genes

Table 2. The 10 genes suppressed by arsenite in 3 human cell types

Accession No.	Name in Unigene database	Symbol
H05891	proliferating cell nuclear antigen	PCNA
R38178	cyclin-dependent kinase 4	CDK4
N75459	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	CCND1
R45031	nucleolar protein 1 (120kD)	NOL1
H16308	immediate early protein	ETR101
R60946	prohibitin	PHB
AA100555	thyroid autoantigen 70kD (Ku antigen)	G22P1
T63504	non-metastatic cells 1, protein (NM23A) expressed in	NME1
R43960	dynein, cytoplasmic, light polypeptide	PIN
H62405	ESTs	--

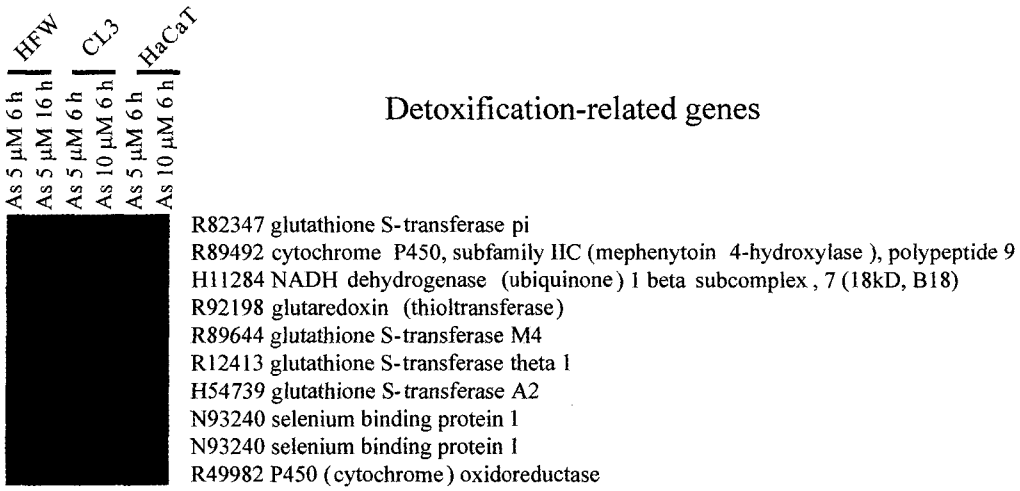

Fig. 3. The expression profiles of detoxification-related genes in arsenite-treated HFW, CL3, and HaCaT cells. HFW were treated with 5 μM arsenite for 6 and 16 h. CL3 and HaCaT cells were treated with 5 and 10 μM for 6 h. After hybridization, color development, and image analysis, the distance of each spot to the regression line was calculated. The distances of each detoxification-related gene to the regression line in each treatment were hierachically clustered. Green colors indicated that the images of spots were near cyan. Red color indicated the images of spots were near magenta.

involved in detoxification in cells treated with arsenite. Figure 3 showed the cluster image of expression profiles of several detoxification-related genes. Glutathione S-transferase (GST) is the cellular key enzyme responsible for metabolic detoxification of numerous toxic compounds produced endogenously or exogenously. Different isoforms of GST were distributed in many tissues (Salinas and Wong, 1999; Hayes and Pulford, 1995). As shown in Fig. 3, the expression of *GST-M4* was elevated in arsenite-treated CL3 and HaCaT cells but not in HFW cells. *GST-θ1* was only induced by arsenite in HaCaT cells. On the other hand, the expression of *GST-π* was suppressed by arsenite in HFW and CL3 cells but without significant change in HaCaT cells. Arsenite did not affect the expression of *GST-A2* in these 3 cell types. These results indicated the pleitrophic effects of arsenite on cellular defense systems. Further investigation is war-

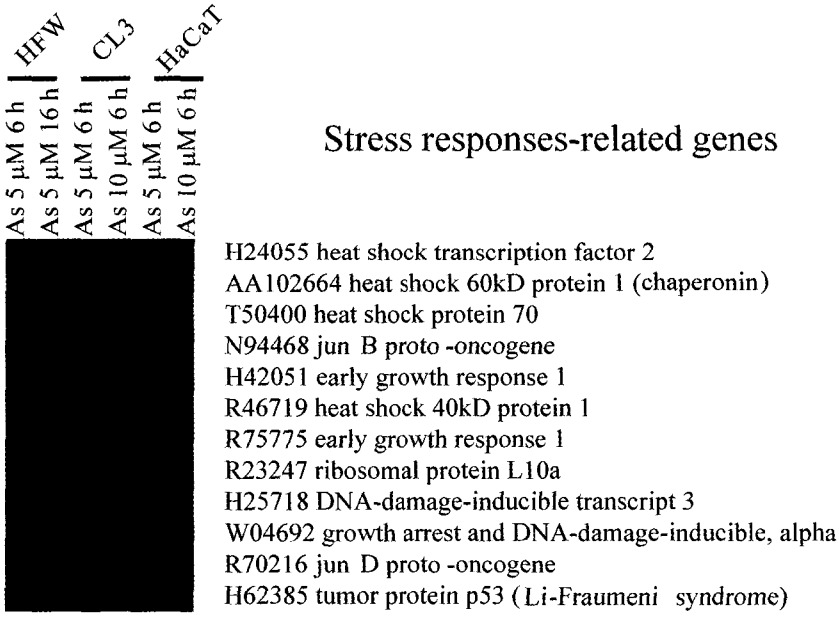

Fig. 4. The expression profiles of stress responded genes in arsenite-treated HFW, CL3, and HaCaT cells. See Fig. 3 for details.

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5. The effects of arsenite on the expression profiles of stress responded genes

As shown in Fig. 4, stress responded genes such as *ribosomal protein L10a*, *GADD 45* and *DDIT 3* were in general enhanced by arsenite in these 3 cell types. The increase expression of *GADD 45* and *GADD 153* by arsenite confirmed that arsenite could induce DNA damages (Lynn *et al.*, 1998; Liu and Jan, 2000) and cell cycle arrest (Yih *et al.*, 1997). The gene product of *GADD153*, also known as product of *DDIT3* or *CHOP-10*, was a member of CCAAT/enhancer binding protein (C/EBP) family (Lekstrom Himes and Xanthopoulos, 1998; Fawcett *et al.*, 1999). GADD153 was undetectable in cells, but highly expressed in cells treated with genotoxic or endoplasmic reticulum stress agents (Welihinda *et al.*, 1999). The roles of GADD153 were closely linked to cell death or cell regeneration (Seth *et al.*, 1999; Zimmermann *et al.*, 2000).

Heat shock protein 70 (*hsp70*) was significantly induced in HFW and CL3 cells treated with arsenite for 6 h, whereas induction of *hsp70* by arsenite in HaCaT was not evident or even slightly suppressed. In addition to *hsp70*, the expression of *hsp60* and *heat shock transcription factor 2* was also significantly enhanced by 5 μM arsenite in HFW cells. The gene products of *jun B* and *jun D* were components of AP-1 which was responsible for trans-activation of genes involved in mitogenic signaling and stress responses. The expression of *jun D* was induced by arsenite in HFW and CL3 cells but not in HaCaT cells. Alternatively, *jun B* was induced in HaCaT cell but suppressed in HFW and CL3 cells.

6. The effects of arsenite on the expression profiles of cell cycle regulatory genes

The expression of genes involved in cell cycle progression was also altered by arsenite treatment (Fig. 5). Arsenite treatment in all three cell lines suppresses the expression of *proliferating cell nuclear antigen*, *cyclin D1*, and *cyclin-dependent kinase 2 (cdk2)*, *cyclin-dependent kinase 4 (cdk4)*, *cdc 2*, and *cdc 27*. These gene products played essential roles in cell cycle progression. On the other hand, the expression of CDK inhibitors, *p21/cip1* was induced in ars-

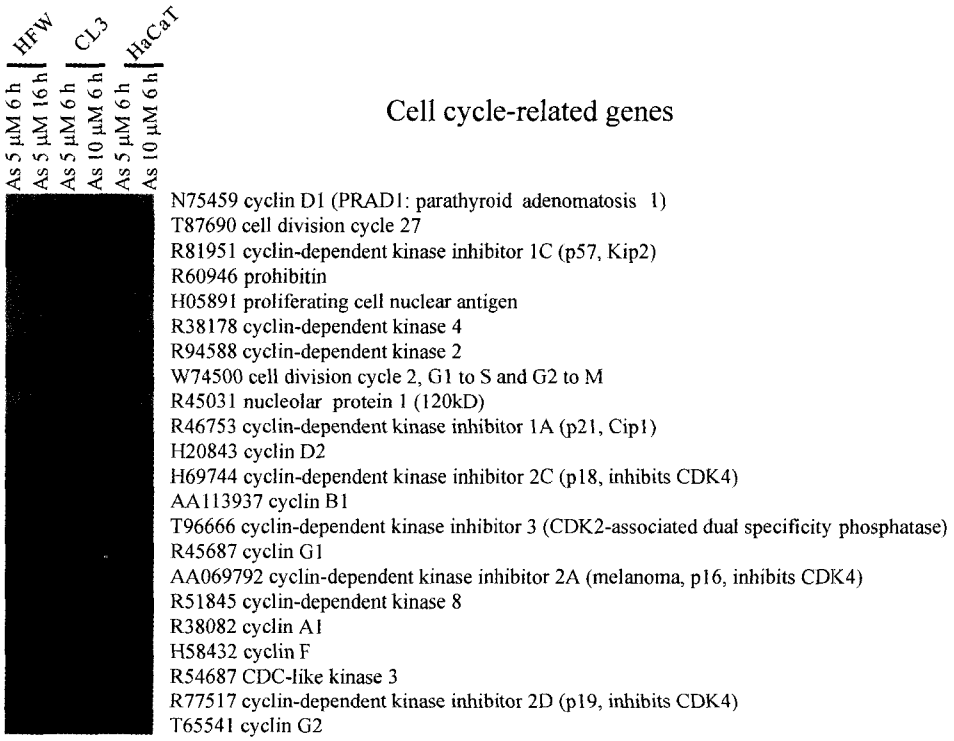

Fig. 5. The expression profiles of cell cycle regulatory genes in arsenite-treated HFW, CL3, and HaCaT cells. See Fig. 3 for details.

enite-treated HFW and HaCaT cells, *p19* was elevated in all three cell lines after arsenite treatment, *p16* was significantly induced in arsenite-treated HFW cells. These results provided evidence showing that the induction of cell cycle arrest by arsenite may mediate its inhibition of CDK or cyclin expression or elevation of CDK inhibitor expression.

7. The effects of arsenite on the expression profiles of transcription factors

The expression of transcription factors involved in global gene expression, including *TATA box binding protein-associated factor, transcription elongation factor B SIII*, and *E2F4* were mainly suppressed in arsenite-treated cells (Fig. 6). However, the expression of transcription factors or transcription regulators involved in expression of stress-response genes, such as *early growth response 1*, *DDIT 3*, *jun B*, *junD*, *cAMP response element-binding protein 1*, and *activating transcription factor 2* (*ATF2*) were generally increased in arsenite-treated cells (Fig. 6).

Egr-1 encodes a zinc finger DNA binding transcription factor and is an immediate-early gene (Liu *et al.*, 1998; Khachigian and Collins, 1998). However, *Egr-1* is inducibly expressed in response to diverse stimuli including mitogenic signals, oxidative stress, and genotoxic stress (Khachigian and Collins, 1998). Egr-1 was able to trans-activate many genes involved in cell growth (such as *transforming growth factor α*, *insulin growth factor II*, *c-myc*, *thymidine kinase*), morphological transformation (*transforming growth factor β1*), and electron transfer (*P450 oxidoreductase*). ATF-2 is a member of the ATF/cAMP-response element-binding protein family of basic region-leucine zipper proteins, which play important roles in the cellular stress response (Fuchs *et al.*, 2000). ATF2 target genes include *tumor necrosis factor α*, *transforming growth factor β*, *cyclin A*, *E-selectin*, *DNA polymerase β*, and *c-jun* (Beier *et al.*, 2000; Zhu and Lobie, 2000). ATF2 has also been reported to up regulate gene expression through a stress-inducible mitogen-activated protein kinase pathway (Wilhelm *et al.*, 1995). Detailed studies regarding the signaling pathway(s) that lead to increased expression of Egr-1, GADD 153, and ATF-2 by arsenite could help unveil the underlying mechanism(s) involved in arsenite toxicity.

8. The effects of arsenite on the expression profiles of p53 downstream genes

As shown in Fig. 7, expression of p53 downstream genes such as *p21/cip1*, *MDM-2*, *GADD 45*, and *cyclin G* were elevated after arsenite treatment, indicating

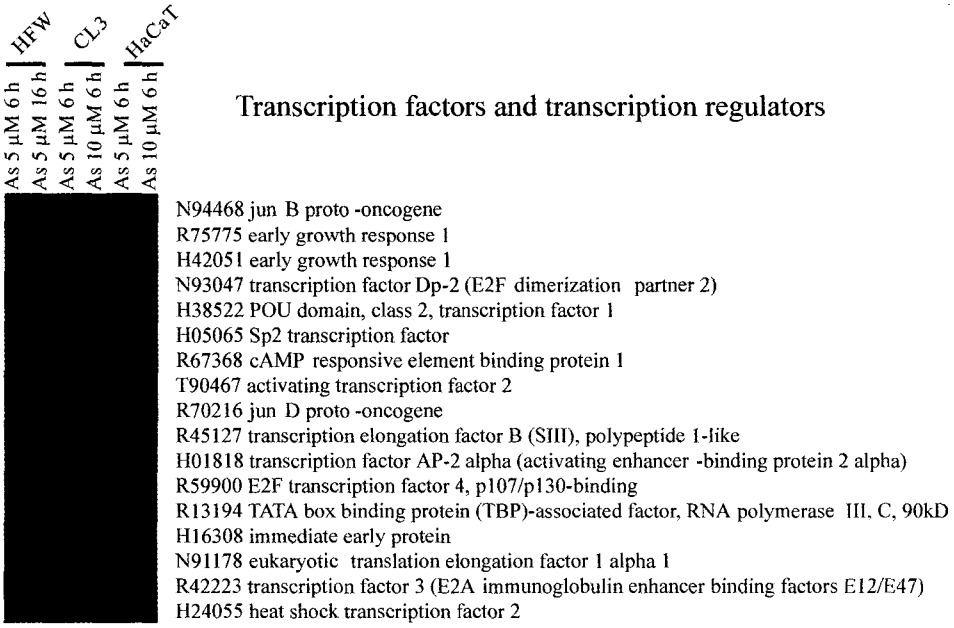

Fig. 6. The expression profiles of transcription factors in arsenite-treated HFW, CL3, and HaCaT cells. See Fig. 3 for details.

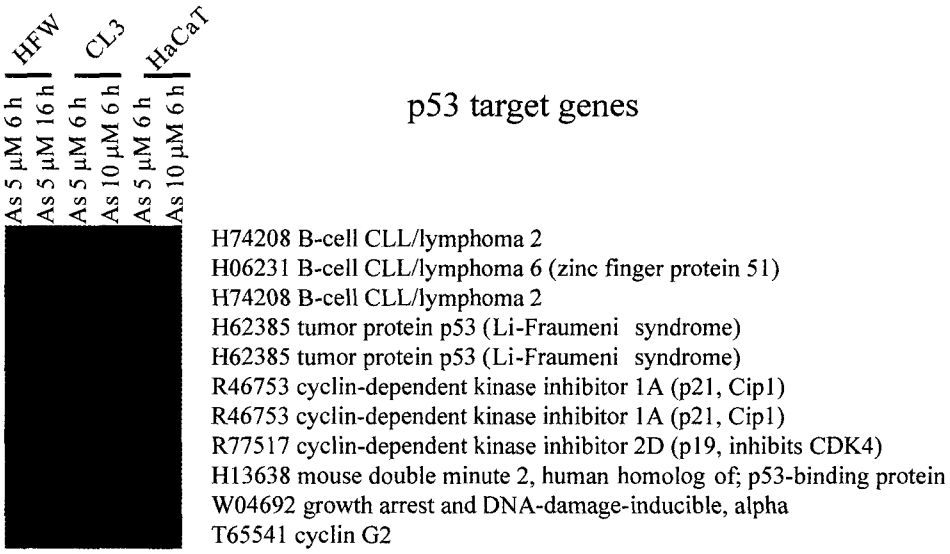

Fig. 7. The expression profiles of p53 downstream genes in arsenite-treated HFW, CL3, and HaCaT cells. See Fig. 3 for details.

that the trans-activation activities of p53 could be activated by arsenite. The *p53* in HFW and CL3 cells is the wild type form. Thus, up-regulation of *MDM-2*, as one of the p53 target genes, in arsenite-treated HFW and CL3 cells was expected. HaCaT cells, with mutant p53, has been reported to induce MDM-2 accumulation after arsenite treatment with unknown reason (Hamadeh *et al.*, 1999).

IV. CONCLUSION

To obtain a more comprehensive picture of pathways associated with arsenite-induced stress responses, colorimetric cDNA microarray was used to study the complex consequences of arsenite insults. In this report, arsenite treatment apparently resulted in increased expression of (i) stress-responded genes including *hsp 70*, *GADD 45*, and *DDIT 3*, (ii) CDK inhibitors including *p21/cip1*, *p16*, and *p19*, (iii) stress response-specific transcription factor genes including *Egr-1*, *jun B* or *jun D*, and *ATF-2*, and (iv) p53 transactivated genes including *cyclin G1* and *MDM-2*. Alternatively, several components involved in cell cycle engine and transcription were inhibited by arsenite treatment. The present results indicated that arsenite could up regulate a specific set of transcription regulators involved in controlling stress responses, proliferation, and genome integrity. However, further investigation was required to unveil the roles of these genes in arsenite toxic effects.

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