Available online at www.sciencedirect.com

ScienceDirect

TOXICOLOGY

Toxicology 239 (2007) 108–115

www.elsevier.com/locate/toxicol

Use of RAPD to detect sodium arsenite-induced DNA damage in human lymphoblastoid cells

Yuan-Cho Lee [a], Vivian.C. Yang [a], Tsu-Shing Wang [b,*]

[a] *Department of Life Science, Tunghai University, Taichung 407, Taiwan*
[b] *Department of Biomedical Sciences, Chung Shan Medical University, Taichung 402, Taiwan*

Received 20 March 2007; received in revised form 23 June 2007; accepted 26 June 2007
Available online 5 July 2007

Abstract

Inorganic arsenic is a known human carcinogen, yet its mechanism of action remains unclear. Our previous study showed that arsenite significantly induces oxidative DNA adducts and DNA–protein cross-links in several mammalian cell lines. In the present study, we used the random amplified polymorphic DNA (RAPD) assay to evaluate the possible target in the genomic DNA of human lymphoblastoid cells that were exposed to sodium arsenite. Treatment with both 10 and 80 μM arsenite for 4 h induced significant changes in RAPD profiles compared with the control pattern. Two 10-mer RAPD primers (D11 and F1) produced the most distinguishable banding profiles between arsenite-treated and control genomic DNA. The sequencing of four arsenite-sensitive RAPD bands showed that the *RB1CC1* and *PACE4* genes might be the DNA targets of sodium arsenite treatment. We propose that arsenite may induce sequence- or gene-specific damage and then change the RAPD profile in human lymphoblastoid cells. The results of our study also show that RAPD combined with other techniques is a good tool for detecting alterations in genomic DNA and for the direct screening of new molecular markers related to arsenite-induced carcinogenesis.
© 2007 Elsevier Ireland Ltd. All rights reserved.

Keywords: RAPD assay; Sodium arsenite; Carcinogenesis

---

1. Introduction

Arsenic poisoning has become a major worldwide environmental concern because as millions of persons have been exposed to excessive arsenic through contaminated drinking water. One of the most important consequences of this exposure is the carcinogenic and atherogenic effect of arsenic in humans. The incidences of tumors among residents in Taiwan in areas where Black-Foot disease is endemic are reported to be higher than those among people in other areas. Oxidative and nitrosative stress was recently postulated as a mode of carcinogenesis of arsenic (Bau et al., 2002; Wang et al., 1996, 1997, 2001). By using the comet assay conjugated with DNA repair enzyme, we successfully showed that sodium arsenite mainly induces oxidative DNA adducts and DNA–protein cross-links in mammalian cells within a pathologically meaningful concentration range (Wang et al., 2001). Although arsenite significantly induced DNA damage at a pathologically or pharmarocologically meaningful concentrations, however, no reports have shown whether arsenite induces site- or gene-specific DNA damage. In the present study, we screened for genome-wide DNA alterations in human lymphoblastoid cells exposed to sodium arsenite by using the random amplified polymorphic DNA (RAPD) method. The RAPD method is a PCR-based technique

---

[*] Corresponding author. Tel.: +886 4 24730022x11811;
fax: +886 4 24757412.
E-mail address: tsushing@csmu.edu.tw (T.-S. Wang).

0300-483X/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2007.06.101

that amplifies random DNA fragments with the use of single short primers of arbitrary nucleotide sequence under low annealing conditions. The technique has been extensively used for species classification and microorganism strain determination. Recently, the RAPD assay was also applied to detect genetic instability in tumors (Papadopoulos et al., 2002) and successfully detected genomic DNA alterations induced by several DNA-damaging agents, such as benzo[*a*]pyrene (Castano and Becerril, 2004; Atienzar and Jha, 2004), heavy metals (Enan, 2006), mitomycin C (Becerril et al., 1999a,b), 4-*n*-nonylphenol and 17-β estradiol (Atienzar et al., 2002), chrysotile asbestos (Yoshida et al., 2001), UV radiation (Kumar et al., 2004; Atienzar et al., 2000) or X-rays, and radio nuclides (Theodorakis et al., 2001). The final purpose of the present work was to identify the possible molecular site or gene-specific markers linked to arsenite-induced carcinogenesis.

2. Materials and methods

2.1. Cell culture and arsenite treatment

Lymphoblastoid cells were transformed by Epstein–Barr virus as described (Wang et al., 2002). One successfully established cell line (N3) from a healthy individual was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 5 ml of 2 mM L-glutamine, and 1% PSN antibiotic mixture at 37 °C in a 5% CO_2 incubator. All media reagents were obtained from GIBCO/Life Technologies (Carlsbad, CA, USA). Cultures were split 1:2 approximately every 3 days to maintain a concentration of 3×10^5 to 1.5×10^6 cells/ml in exponential growth. The sodium arsenite purity 99% (Sigma, MO) was dissolved in water, sterilized by membrane filtration, and stored at −20 °C until used. The growing N3 cells were centrifuged and the cell pellet was resuspended in complete RPMI 1640 medium (5×10^5 cells/ml). Different concentrations of sodium arsenite were added to the culture for 4 h at 37 °C. After incubation, the cells were washed with PBS and collected by centrifugation for further DNA, RNA, and protein isolation.

2.2. Genomic DNA extraction and RAPD analysis

Total DNA from the N3 cells was extracted and purified by using a DNeasy tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The concentration of DNA was determined by spectrophotometry. Ninety arbitrary primers (Operon Technologies, Southampton, UK) were used for RAPD analysis. The DNA samples were then diluted to 2.5 ng/μl for RAPD analysis. The PCR amplifications were performed in 30 μl of reaction mixture containing 3 μl of 10× enzyme buffer, 3 μl of 2 μM dNTP, 2 μl of 5 pmol random primer, 1.5 U of *Taq* DNA polymerase, and 25 ng of DNA as the template. The PCR conditions were as follows: denaturing at 94 °C for 0.5 min, annealing at 40 °C for 1 min, and extension at 72 °C for 1 min. The PCR products (20 μl each) mixed with loading buffer were loaded in 2% agarose gels and electrophoresed at 100 V for 70 min. The gels were stained with ethidium bromide (0.5 μg/ml) and visualized under UV light.

2.3. Cloning and sequencing of RAPD products

Amplified fragments were excised from the agarose gels and DNA was eluted. The eluted DNA was reamplified with the same random primer. The reamplified DNA fragments were then cloned by using a TA cloning kit (Invitrogen Taiwan, Ltd) according to the protocol provided by the manufacturer. The recombinant plasmid DNA was digested with EcoRI to confirm the insert size. The cloned RAPD-PCR product was sequenced by use of a DNA sequencing machine. Sequences were compared with known sequences in the GenBank database by using the BLASTn and BLASTx programs (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA).

2.4. Southern hybridization

RAPD-PCR products were transferred from agarose gels onto Hybond-N nitrocellulose membranes (Amersham–Pharmacia Tech., Baie d'Urfe, Quebec) according to Sambrook et al. (1989). The D11b fragment used as a probe was generated by PCR and labeled with α-[^{32}P]dATP by using a Random Primer DNA labeling kit (Invitrogen Canada Inc., Burlington, ON) according to the manufacturer's protocol. After hybridization, the blot was washed in 2× SSC containing 0.1% SDS twice for 20 min each and in 1× SSC containing 0.1% SDS once at room temperature and exposed to X-ray film for autoradiography at −80 °C with an intensifying screen for 4–6 days.

3. Results

3.1. Effect of sodium arsenite-modified DNA on RAPD profiles

For the RAPD analysis, 90 random 10-mer primers (Table 1) were used to amplify genomic DNA samples from arsenite-treated and untreated N3 cells. A total of 85 of 90 (94%) RAPD primers generated strong banding patterns in all samples tested; the other 5 primers (6%) failed to amplify DNA, probably as the result of PCRs or temperature conditions. A total of 814 amplification products (loci) from the 85 primers were identified, with an average of 9.6 products per primer. The total number of products amplified by an individual primer ranged from 4 for the D3 and D5 primers to 18 for the G4 primer (Table 1). From the 90 random 10-mer primers, 10 frequently generated RAPD profile variations were seen in the arsenite-treated

Table 1
The sequences and the resulting bands of some representing primers used for RAPD amplification of arsenite-treated and control genomic DNA from normal human lymphoblastoid cells

Primer	Sequense (5′ → 3′)	Total bands	Primer	Sequence (5′ → 3′)	Total bands
A1	CAGGCCCTTC	Smear	C4	GTCCACACGG	7
B1	CAGCACCCAC	Smear	C6	CTGCTGGGAC	11
E11	GTTGCCAGCC	Smear	D1	AGGGAACGAG	7
C2	TGCTCTGCCC	Smear	D3	ACCCCCGAAG	4
A2	TGCCGAGCTG	0	D5	TTCGAGCCAG	4
A9	GGGTAACGCC	11	D11	GTCCCGACGA	8
A11	CAATCGCCGT	6	F1	ACCGCGAAGG	10
B9	GTTTCGCTCC	9	F7	TTGGCACGGG	11
C1	TGCGCCCTTC	15	G4	AGGGCGTAAG	18
C3	GGTGACGCAG	9			

genomic DNA samples compared with the untreated control. Furthermore, 2 primers, designated D11 (5′-GTCCCGACGA-3′) and F1 (5′-ACCGCGAAGG-3′), produced reproducible and the most distinguishable banding profiles between arsenite-treated and untreated samples after at least five independent RAPD assays (Fig. 1). By contrast, most of the 10-mer primers gave band patterns of almost the same intensity, for example, C1 and F7 (data not shown).

The RAPD band patterns produced by the D11 and F1 primers were recorded by photography and quantified by using Scion Image software (National Institutes of Health, Bethesda, MD, USA). Figs. 2 and 3 give an overview of the RAPD band patterns obtained with the D11 and F1 primers in three independent experiments. In total, 35 bands and 42 bands were detected in the three independent RAPD-PCR analyses with the D11 and F1 primers, respectively. The peak maximum pixel-intensity ratios of sodium arsenite-treated to control RAPD band patterns with the D11 and F1 primers are shown in Table 2. The ratio ranged from 0.68 to 1.82 with the D11 primer and from 0.67 to 1.84 with the F1 primer. Treatment for 4 h with both 10 and 80 μM sodium arsenite could increase or decrease the specific band intensity of the RAPD patterns with the D11 and F1 primers, and 83.2% of the specific band intensity of the RAPD pattern was changed by sodium arsenite treatment Table 3. Among these arsenite-susceptible RAPD bands, seven RAPD bands (#15, #20, #34 in the D11 primer group and number #3, #4, #9, #12 in the F1 primer group) showed a dose-response relation with arsenite doses (Figs. 2 and 3, and Table 2.

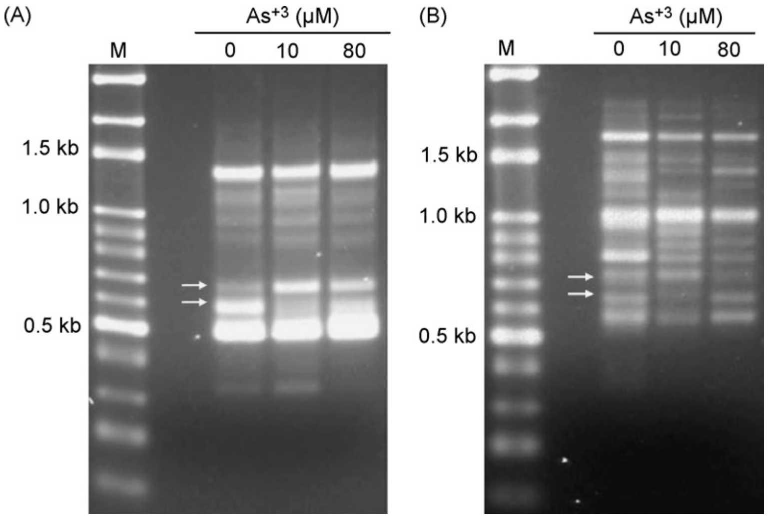

Fig. 1. An example of the typical RAPD banding pattern observed in genomic DNA from untreated and sodium arsenite-treated human lymphoblastoid cells. Human lymphoblastoid cells were exposed to 10 or 80 μM sodium arsenite for 4 h; thereafter, DNA was isolated and quantified and used as the template in the RAPD-PCR analysis. The RAPD patterns were obtained by using the D11 (A) and F1 (B) primers. The RAPD bands selected for isolation, cloning, and sequencing are indicated by arrows. M refers to 100 bp DNA markers.

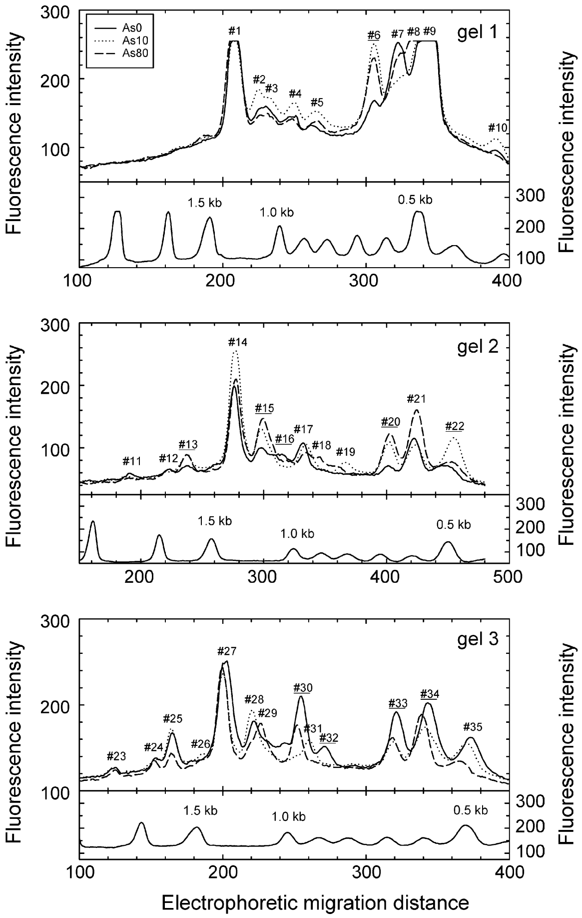

Fig. 2. Detection and quantitation of RAPD banding patterns produced by the D11 primer. The PCR products were separated on 1.5% agarose gels that were stained with ethidium bromide. Photographs of gels were analyzed by using Scion Image software. The underlined numbers in the RAPD profile indicate that the band intensities were increased or decreased significantly in the two treatments of sodium arsenite.

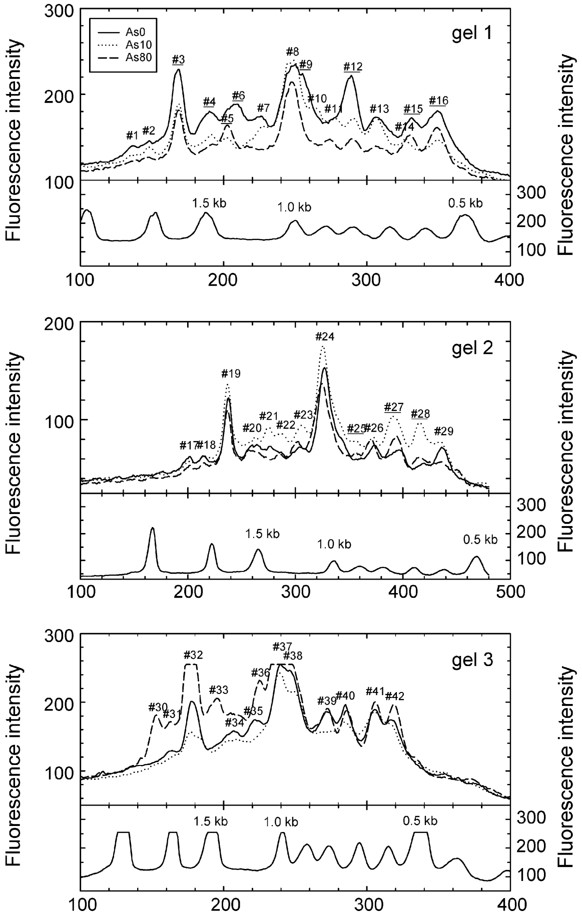

Fig. 3. Detection and quantitation of RAPD banding patterns produced by the F1 primer. The PCR products were separated on 1.5% agarose gels that were stained with ethidium bromide. Photographs of gels were analyzed by using Scion Image software. The underlined numbers in the RAPD profile indicate that the band intensities were increased or decreased significantly in the two doses of arsenite treatments.

3.2. Cloning, sequencing, and sequence homology searching of the arsenite-specific RAPD bands amplified by the D11 and F1 primers

Four arsenite-specific RAPD bands (D11-a, D11-b, F1-a, and F1-b) amplified with the D11 or F1 primer were further cloned and sequenced (Table 4). Sequence homology searches revealed that the D11-b and F1-a amplified fragments had highly sequence similarity (99% identity) with the *RB1CC1* DNA clone, Accession No. AC113139.2, and the *PACE4* DNA clone, Accession No. AC023024.6, respectively (Table 4). The other two bands could not be matched to known genes on the basis of their chromosome localization. When the D11-b DNA fragment was used as a DNA probe to hybridize with the RAPD-PCR DNAs amplified with the D11 primer, only one specific band was produced. The amplification levels significantly decreased in the present of sodium arsenite (Fig. 4). The Southern blot data confirmed that the *RB1CC1* gene was a genomic target of sodium arsenite treatment.

4. Discussion

This is the first report of an analysis of genomic alterations in arsenite-treated human lymphoblastoid cells with the use of RAPD-PCR fingerprinting. Eighty-three percent of the RAPD bands were changed by sodium arsenite treatment. These data prove that the RAPD-PCR method is useful for the screening and characterization of genomic regions that have undergone alterations as the result of arsenite treatment. Changes in the genome that

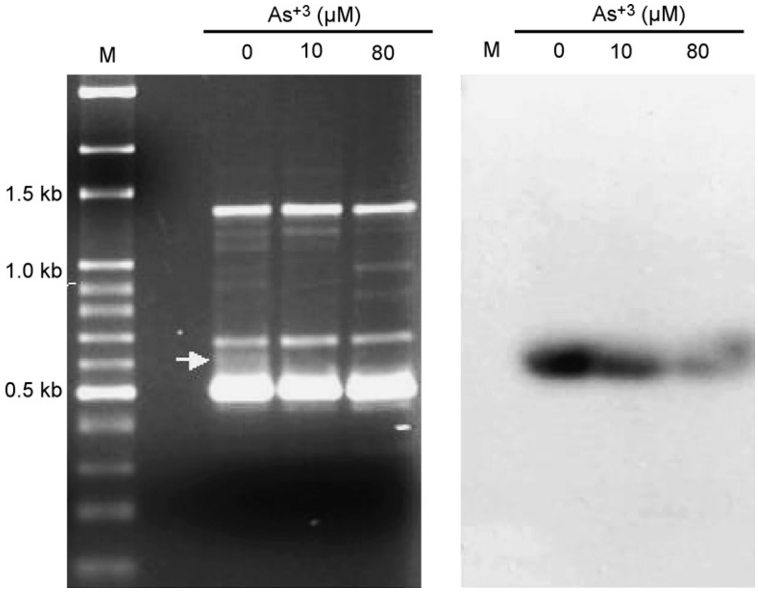

Fig. 4. Southern blot of the RAPD pattern obtained with the D11-b probe. Genomic DNA from untreated and sodium arsenite-treated human lymphoblastoid cells was amplified with D11 primers, and the PCR products were transferred onto a nylon membrane and hybridized with the D11-b probe. M refers to 100 bp DNA markers.

Table 2
The peak maximum fluorescence intensity ratio of RAPD bands increased or decreased significantly in the two treatments of sodium arsenite relative to control with the D11 and F1 primers

Band No.	D11			Band No.	F1		
	As10/As0	As80/As0	Pattern*		As10/As0	As80/As0	Pattern*
6	1.49	1.36	+4 + 3	3	0.82	0.79	−1 − 2
13	1.24	1.25	+2 + 2	4	0.84	0.78	−1 − 2
15	1.28	1.49	+2 + 4	5	0.81	0.90	−1 − 1
16	0.80	0.90	−2 − 1	6	0.73	0.76	−2 − 2
20	1.55	1.75	+5 + 7	9	0.88	0.74	−1 − 2
22	1.82	1.18	+8 + 1	12	0.78	0.67	−2 − 3
30	0.68	0.77	−3 − 2	15	0.83	0.89	−1 − 1
32	0.86	0.85	−1 − 1	16	0.81	0.89	−1 − 1
33	0.80	0.80	−2 − 2	25	1.31	1.10	+3 + 1
34	0.84	0.80	−1 − 2	27	1.61	1.20	+6 + 2
				28	1.84	1.16	+8 + 2

[*] Comparing the RAPD band fluorescence intensity with the control one, the value of the pattern is added to or subtracted by 1 for every 0.1 increase or decrease, respectively. The value of the pattern is zero whether the band fluorescence intensity increases or decreases comparing to the control between ±0.1. The first number in column "pattern" relates to As10/As0, and the second to As80/As0.

Table 3
Arsenite-induced changes in band intensity patterns compared with the untreated control

Pattern	Unchanged	Two increases/decreases[a]	One increase/decrease[b]	Combination of increase and decrease[c]	Total band
D11	8	10	16	1	35
F1	5	11	20	6	42
Sum and ratio	13 (16.8%)	21 (27.3%)	36 (46.8%)	7 (9.1%)	77

[a] Both arsenite treatments (10 and 80 μM) were increased or decreased in the individual RAPD band intensity.

[b] Only one dose of arsenite treatment increases or decreases the individual RAPD band intensity.

[c] Two arsenite treatments induced a one decrease and one increase in RAPD band intensity, respectively.

Table 4
Sequence analysis of four specific RAPD bands that are sensitive to sodium arsenite treatment

Clone	Fragment size (bp)	NCBI definition	Localization	Gene
D11-a	591	RP11-162A23	Chr. 10	Unknown
D11-b	497	RP11-1152K2	Chr. 08	RB1CC1 intron 20
F11-a	665	RP11-299G20	Chr. 15	PACE4 intron 12
F11-b	491	.B780G18	Chr. 21	Unknown

were observed in the present study were mainly variations in RAPD band intensity in the profiles generated by sodium arsenite-exposed N3 cells. We seldom saw new bands or loss of bands after sodium arsenite treatment. These results suggest that short-term (4 h) sodium arsenite treatment induces mainly DNA damage, which causes the specific RAPD band intensity to either increase or decrease.

Short-term treatment with sodium arsenite did not seem to induce permanent genomic mutations or changes in oligonucleotide priming sites that would mainly produce new or result in lost RAPD bands. Our previous research showed that sodium arsenite mainly induces oxidative DNA damage and DNA–protein cross-links (Wang et al., 2002). The influence of different types of DNA damage on primer pairing and *Taq* polymerase extension is still unclear. A recent report showed that a single 8-oxo-7′, 8′-dihydro-2′-deoxyadenosine lesion, a basic site, or a T–T dimer dramatically reduced amplification efficiency (Sikorsky et al., 2004). In addition to DNA damage type, the relative position of the DNA lesions within the template also influences polymerase progression. For example, a single 8-oxodG did not significantly perturb amplification, but two tandem 8-oxodGs did (Sikorsky et al., 2004). These findings suggest that sodium arsenite-induced oxidative DNA damage and DNA–protein cross-links might block *Taq* polymerase processing and decrease the intensity of some specific RAPD bands produced by the D11 or F1 primer.

Besides decreasing the intensity of specific RAPD bands, arsenite treatment also significantly increased the intensity of RAPD bands produced with the D11 and F1 primers. In fact, this phenomenon was also found by other researchers (Liu et al., 2005). We thought that it would be impossible to enhance the enzyme activity of amplification with *Taq* polymerase by arsenite-induced DNA damage. Arsenite-induced DNA modifications enhancing the pairing activity of the primer with the DNA template may be a possible explanation for the increase in intensity of some RAPD bands. The influence of arsenite-induced DNA damage on DNA synthesis, including blocking of *Taq* polymerase and pairing of random 10-mer primers, will require further study to clarify. Nevertheless, the results of our study strongly suggest that arsenite-induced genomic DNA damage and alterations are reflected by the RAPD-PCR method.

It is interesting to note the change of RAPD band patterns do not show a dose-dependently tendency to arsenite in this study. Recently, several similar findings have been reported by Liu et al. (2005) and Castano and Becerril (2004) who used RAPD-PCR to analyze the genotoxicant-induced DNA damage. This phenomenon is probably due to RAPD-PCR is a method for qualification rather than for quantitation for DNA damage assay. In fact, the nature and amount of DNA impact in RAPD band can only be obtained by sequencing or probing (Atienzar and Jha, 2006). Besides the limit of RAPD-PCR, 4 h arsenite exposure may also be not a good time point to induce a relatively stable of DNA alternations. This point of view can be supported by Beceril (1999) who reported the consistency of the changes of RAPD-band patterns depends on the exposure period of mitomycin C.

In the present study, we screened ninety 10-mer primers and found two primers (D11 and F1) to be most informative. We compared the sequence of these primers with other informative primers used in other RAPD-PCR studies, and discovered that our primers have not been used in other studies to reflect the genomic alterations induced by DNA-damaging agents (Table 5). However, the A9, B9, C1, C2, C3, C4, C6, and D1 primers have been used to reflect genomic alterations induced by benzo[*a*]pyrene (Atienzar and Jha, 2004); the A11 primer has been used to reflect genomic alterations induced by azoxymethane (Luceri et al., 2000). The report by Becerril et al. (1999a,b) showed that the primer 5′-GATCCATTGC is more informative for mitomycin C- and benzo[*a*]pyrene-induced genomic alterations in a fish cell line. However, Atienzar and Jha (2004) used nine random 10-mer primers to detect genomic alterations induced by benzo[*a*]pyrene in water fleas that were completely different from the primers Castano and Becerril (2004) used. These results imply that the primers that reflect genomic alterations induced by DNA-damaging agents

Table 5
Comparison of primers used for the RAPD technique to detect toxicant-induced genomic change

Primer (5′–3′)	Chemical	Cell type	Author
GATCCATTGC GGGCCTCTGAT AGCACTGTCA CCAAGTCGACA	Benzo[*a*]pyrene	Fish cell line	Castano and Becerril (2004)
AGCGGCCATT	UV-B	Cyanobacteria	Kumar et al. (2004)
GGGTAACGCC (A9) GTTTCGCTCC (B9) TGCGCCCTTC (C1) TGCTCTGCCC (C2) GGTGACGCAG (C3) GTCCACACGG (C4) CTGCTGGGAC (C6) AGGGAACGAG (D1) GTAGACCCGT	Benzo[*a*]pyrene	*Water fleas*	Atienzar and Jha (2004)
TACACTAGCG CAATCGCCGT (A11) AACGCGCAAC TGCGCGATCG CACCTGCCGC GCACGCCGGA	Azoxymethane	Rat colon tumors	Luceri et al. (2000)
GATCCATTGC	Mitomycin C	Fish cell line	Becerril et al. (1999a,b)
GTCCCGACGA (D11) ACCGCGAAGG (F1)	Sodium arsenite	Lymphoblastoid cells	This study

may act in cell- or species-specific and agent-specific manners. Although we cannot exclude that other random 10-mer primers may be better than the D11 and F1 primers for reflecting genomic alterations induced by sodium arsenite, these primers may help us to identify targets of arsenite-induced genomic alterations. The specificity of the D11 and F1 primers for detecting arsenite-induced genomic damage needs further study. To further clarify their specificity, we are currently using the D11 and F1 primers to analyze genomic alterations induced by different DNA-damaging agents, including arsenite, cadmium, bezo[*a*]pyrene, and mitomycin C.

To explore the possible genomic targets for arsenite-induced DNA damage, we isolated four RAPD bands for further cloning and sequencing. Two of these perfectly matched the introns of the human *RB1CC1* and *PACE4* genes; the other two bands did not match to a known gene (Table 4). The lower repair efficiency of intron and noncoding regions of DNA may be the reason all four isolated RAPD-PCR bands were matched to them. *RB1CC1*, a novel human gene, can induce RB1 in various human cells and suppresses cell cycle progression through RB1 expression in human neoplastic cells. *RB1CC1* is frequently mutated in breast cancer and shows characteristics of a classic tumor-suppressor gene (Chano et al., 2002). *PACE4*, a member of the family of proprotein convertases, was shown to enhance mouse skin carcinoma progression by increasing tumor cell invasiveness. Although both *RB1CC1* and *PACE4* are important regulators for carcinogenesis, we could not conclude that these two genes are the genomic targets of arsenite exposure on the basis of the limited sequencing data of the RAPD bands in the present study. To establish more reliable genomic targets, further systematic sequencing of the RAPD bands that are specifically modulated by sodium arsenite will be necessary.

In conclusion, the RAPD-PCR method can be used as an investigational tool for sodium arsenite-induced genomic alterations. Furthermore, the present results suggest that RAPD-PCR fingerprinting in conjugation with molecular cloning and gene sequencing can be a powerful screening tool for searching for specific genomic targets that are attacked by arsenite exposure. We found two informative 10-mer primers, D11 and F1, that may have great potential for detecting arsenite-induced specific genomic alterations.

Acknowledgements

This work was partially supported by the National Research Council of ROC under NSC94-2314-B-040-028.

References

Atienzar, F.A., Jha, A.N., 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat. Res. 613, 76–102.

Atienzar, F.A., Jha, A.N., 2004. The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B[*a*]P exposed Daphnia magna. Mutat. Res. 552, 125–140.

Atienzar, F.A., Billinghurst, Z., Depledge, M.H., 2002. 4-*n*-Nonylphenol and 17-beta estradiol may induce common DNA effects in developing barnacle larvae. Environ. Pollut. 120, 735–738.

Atienzar, F.A., Cordi, B., Donkin, M.E., Evenden, A.J., Jha, A.N., Depledge, M.H., 2000. Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae Palmaria palmata. Aquat. Toxicol. 50, 1–12.

Becerril, C., Ferrero, M., Sanz, F., Castano, A., 1999a. Detection of mitomycin C-induced genetic damage in fish cells by use of RAPD. Mutagenesis 14 (5), 449–456.

Bau, D.T., Wang, T.S., Chung, C.H., Wang, A.S., Wang, A.S., Jan, K.Y., 2002. Oxidative DNA adducts and DNA–protein cross-links are the major DNA lesions induced by arsenite. Environ. Health Perspect. 110 (Suppl. 5), 753–756.

Becerril, C., Ferrero, M., Sanz, F., Castano, A., 1999b. Detection of mitomycin C-induced genetic damage in fish cells by use of RAPD. Mutagenesis 14, 449–456.

Castano, A., Becerril, C., 2004. In vitro assessment of DNA damage after short- and long-term exposure to benzo[*a*]pyrene using RAPD and the RTG-2 fish cell line. Mutat. Res. 552, 141–151.

Chano, T., Kontani, K., Teramoto, K., Okabe, H., Ikegawa, S., 2002. Truncating mutations of RB1CC1 in human breast cancer. Nat. Genet. 31, 285–288.

Enan, M.R., 2006. Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol. Appl. Biochem. 43, 147–154.

Kumar, A., Tyagi, M.B., Jha, P.N., 2004. Evidences showing ultraviolet-B radiation-induced damage of DNA in cyanobacteria and its detection by PCR assay. Biochem. Biophys. Res. Commun. 318, 1025–1030.

Liu, W., Li, P.J., Qi, X.M., Zhou, Q.X., Zheng, L., Sun, T.H., Yang, Y.S., 2005. DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere 61, 158–167.

Luceri, C., De Filippo, C., Caderni, G., Gambacciani, L., Salvadori, M., Giannini, A., Dolara, P., 2000. Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. Carcinogenesis 21, 1753–1756.

Papadopoulos, S., Benter, T., Anastassiou, G., Pape, M., Gerhard, S., Bornfeld, N., Ludwig, W.D., Dorken, B., 2002. Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. Int. J. Cancer 99, 193–200.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Sikorsky, J.A., Primerano, D.A., Fenger, T.W., Denvir, J., 2004. Effect of DNA damage on PCR amplification efficiency with the relative threshold cycle method. Biochem. Biophys. Res. Commun. 323, 823–830.

Theodorakis, C.W., Bickham, J.W., Lamb, T., Medica, P.A., Lyne, T.B., 2001. Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada Test Site USA. Environ. Toxicol. Chem. 20, 317–326.

Wang, T.S., Hsieh, L.J., Hsu, T.Y., Chung, C.H., Li, S.Y., 2002. DNA damage and repair in lymphoblastoid cell lines from normal donors and fragile X syndrome patients. Arch. Med. Res. 33, 128–135.

Wang, T.S., Hsu, T.Y., Chung, C.H., Wang, A.S., Bau, D.T., Jan, K.Y., 2001. Arsenite induces oxidative DNA adducts and DNA–protein cross-links in mammalian cells. Free Radic. Biol. Med. 31, 321–330.

Wang, T.S., Kuo, C.F., Jan, K.Y., Huang, H., 1996. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J. Cell. Physiol. 169, 256–268.

Wang, T.S., Shu, Y.F., Liu, Y.C., Jan, K.Y., Huang, H., 1997. Glutathione peroxidase and catalase modulate the genotoxicity of arsenite. Toxicology 121, 229–237.

Yoshida, N., Naka, T., Sengoku, T., Ogawa, K., 2001. Toxicity and mutagenesis of chrysotile asbestos to Agrobacterium radiobacter. Curr. Microbiol. 42, 398–402.