Arsenic-Related Chromosomal Alterations in Bladder Cancer

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Background: **Previous studies have demonstrated that ingestion of arsenic in drinking water is a strong risk factor for several forms of cancer, including bladder cancer. It is not known whether arsenic-related cancers are genetically similar to cancers in unexposed individuals or what mechanisms of carcinogenesis may underlie their formation. This study was designed to compare chromosomal alterations in bladder cancers of arsenic-exposed individuals to provide insight into the mechanism of how arsenic may induce or promote cancer.** ***Methods:*** **A case–case study was conducted in Argentina and Chile examining chromosomal alterations in bladder tumor DNA in 123 patients who had been exposed to arsenic in their drinking water. Patients were placed into one of four arsenic exposure categories according to their average 5-year peak arsenic exposure. Patients were also classified as ever smokers or never smokers. Comparative genomic hybridization was used to identify chromosomal alterations throughout the genome. All statistical tests were two-sided.** ***Results:*** **The total number of chromosomal alterations was higher in individuals exposed to higher arsenic levels (5.7 ± 5.1, 5.6 ± 5.1, 7.3 ± 7.4, and 9.1 ± 6.5 [mean ± standard deviation] chromosomal alterations per tumor with increasing arsenic exposure; P_{trend} = .02, adjusted for stage and grade). The trend was stronger in high-grade (G2–G3) tumors (6.3 ± 5.5, 8.3 ± 4.7, 10.3 ± 7.8, and 10.5 ± 6.4 alterations per tumor; P_{trend} = .01) than it was in low-grade (G1) tumors (3.5 ± 3.1, 1.1 ± 1.1, 2.5 ± 2.5, and 3.6 ± 3.2 alterations per tumor; P_{trend} = .79). The mean number of chromosomal alterations also increased with tumor stage and grade (P_{trend}<.001) independently of arsenic exposure but was not associated with smoking history. Deletion of part or all of chromosome 17p (P_{trend}<.001) showed the strongest association with arsenic exposure.** ***Conclusions:*** **Bladder tumors in patients with higher levels of arsenic exposure showed higher levels of chromosomal instability. Most of the chromosomal alterations associated with arsenic exposure were also associated with tumor stage and grade, raising the possibility that bladder tumors from arsenic-exposed patients may behave more aggressively than tumors from unexposed patients. [J Natl Cancer Inst 2002;94:1688–96]**

Exposure to arsenic is considered a major public health concern, particularly because of its clear carcinogenic potential *(1,2).* However, the molecular mechanism by which and the dose at which it causes cancer are still unclear.

Humans are environmentally exposed to arsenic primarily through drinking water, with chronic exposure being an established cause of dermatologic effects and skin cancer *(1).* However, epidemiologic evidence from the Blackfoot endemic region of Taiwan suggests that arsenic exposure causes internal cancers that are more fatal than skin cancers, including those of the bladder, kidney, liver, and lung *(3–7).* The cancer for which long-term arsenic exposure produces the greatest risk is bladder cancer: estimated mortality risk ratios in the most highly arsenic-exposed population (i.e., with drinking water containing arsenic concentrations >600 μg/L with a weighted average of 800 μg/L) were 28.7 for men and 65.4 for women *(5,8).* Studies in South American populations have found increased relative risks for bladder cancer in areas with high arsenic concentrations in drinking water. For example, in Northern Chile, 400 000 people were exposed to approximately 600 μg/L of naturally occurring arsenic in drinking water from 1955 through 1969 *(9).* Bladder cancer mortality was markedly elevated in this population, with standardized mortality ratios (SMRs) of 6.0 (95% confidence interval [CI] = 4.5 to 7.4) for men and 8.2 (95% CI = 6.3 to 10.5) for women. An ecologic study conducted in Argentina also detected increased bladder cancer mortality in arsenic-exposed individuals *(10).* Bladder cancer relative risks in the highest arsenic-exposed areas were 2.1 (95% CI = 1.8 to 2.5) for men and 1.8 (95% CI = 1.2 to 2.6) for women.

The molecular mechanism by which arsenic promotes cancer is not yet known, nor is the dose at which it causes cancer. Studies comparing genetic alterations in tumors from arsenic-exposed and unexposed individuals may provide insight into the mechanism(s) of arsenic-induced cancers. One technique, comparative genomic hybridization (CGH), can be used to identify genetic alterations throughout the genome. CGH allows assessment of alterations in the relative copy number of DNA sequences, which are detected by simultaneously hybridizing tumor DNA extracted from clinical tumor samples and normal tissue or lymphocyte DNA onto normal metaphase spreads. Unlike some other methods of genetic analysis of tumors, CGH can be performed with tumor DNA extracted from archival paraffin blocks. CGH can be used as a tool to locate possible regions of genetic imbalance in tumor cells, which can then be analyzed at higher resolution by using techniques such as allelotype analysis or CGH microarrays.

In this article, we present findings from a case–case study comparing genetic changes in bladder tumors from individuals exposed to high and low levels of arsenic in Argentina and

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See "Notes" following "References."

Chile. DNA in tumors from bladder cancer patients exposed to a range of arsenic concentrations in their drinking water were compared to identify chromosomal copy number changes associated with arsenic exposure. In addition to arsenic exposure, patients were also stratified by tumor stage, tumor grade, sex, and smoking history to determine associations of these possible confounders with the frequencies and types of chromosomal alterations observed.

Subjects and Methods

Patient Selection and Interviews

These studies were reviewed and approved by the University of California, Berkeley, Committee for Protection of Human Subjects, and written informed consent was obtained from each subject. The study included all 94 patients who were newly diagnosed with transitional cell carcinoma (TCC) of the bladder between 1996 and 2000 who resided in Union County in the Province of Cordoba, Argentina (population 95 220; 1991 census). This county was selected because high bladder cancer mortality relative risks were previously seen there, and a wide range of arsenic levels were known to exist in the drinking water (range = 40–533 μg/L) *(10)*. Bladder cancer patients were identified by urologists and pathologists. Patients were interviewed in their homes about their lifetime residential history, current and past drinking water consumption, smoking history, and occupational history. All bladder cancer tumors were verified to be transitional cell carcinomas (TCC) by histologic review (O. A. Rey, V. Bhargava).

The study also included 29 patients with TCC who were ascertained from a hospital-based case–control study previously conducted in regions I, II, and III of Northern Chile that included individuals exposed to very high concentrations (>500 μg/L) of arsenic in their drinking water. Selection of Chilean bladder cancer case patients has been described elsewhere *(11)*. Briefly, nurses identified bladder cancer patients in the public hospitals between November 1994 and July 1996. TCC had to be confirmed by biopsy, and the patients' first diagnosis had to have occurred either during or no more than 1 year before their current hospital admission. The majority of Chilean patients (70%) were interviewed while still in the hospital. The remaining 30% of patients were visited and interviewed in their homes after discharge. Information about the patient's lifetime residential history, current and past drinking water consumption, smoking history, and occupational history was collected at the time of interview.

Arsenic Exposure Assessment

Arsenic exposure for each individual was determined by measuring arsenic concentrations in the drinking water at their current and previous residences. Subjects from Argentina drank water from various sources, including public water supplies, private wells, bottled sources, and aljibes (a type of underground storage installation in which rain water is collected and stored). To calculate arsenic concentrations in public water supplies, arsenic measurements in drinking water were collected from public records dated from 1960 through 2001. Information on the exact location of each private well was collected from each patient at the time of interview. If a well was closed or could not be located, a proxy measurement of arsenic concentration was determined from the water obtained from the nearest well of similar depth. Water samples were frozen in 50-mL aliquots and brought to the University of Washington, Seattle, where arsenic was measured by hydride generation according to previously published methods *(12)*. Bottled and aljibe water were considered free of arsenic contamination. Unlike well water, aljibe water consists of rainwater and, in general, is not contaminated with arsenic, although it could be contaminated if the aljibe wall was cracked and contaminated ground water entered and mixed with the rain water. To ensure that external contamination was not a problem, water from many aljibes was tested for arsenic, and arsenic contamination was not found. The same test was performed on several types of bottled waters, and they also were found free of arsenic contamination.

In Chile, almost 100% of urban households are served by public water supplies and, overall, the large majority of the Chilean population receives water from either town or city supplies. Since 1950, water supply companies have measured the concentration of arsenic in drinking water at least once per year and more frequently after 1970. In this study, we collected data on arsenic concentrations from 1950 through 1994. Concentrations in earlier years, between 1930 and 1950, were estimated based on measurements from 1950.

All 123 patients were grouped into one of four arsenic exposure categories based on the average of the 5 years of highest arsenic exposure in their drinking water. This value was calculated using residential histories and the concentration of arsenic at each location of residence occurring between 6 and 40 years ago. Categories 1–4 consisted of tumors from patients whose average 5-year peak arsenic concentration in their drinking water was as follows: category 1, between 0 and <10 μg/L per year (n = 45; this category included patients from both Chile and Argentina); category 2, 10–99 μg/L per year (n = 24); category 3, 100–299 μg/L per year (n = 29); and category 4, ⩾300 μg/L per year (n = 25). Most of those patients in the highest exposure category were from region II of Chile. The cut points for these arsenic exposure categories were chosen because category 1 is the arsenic concentration in water (<10 μg/L) that the U.S. Environmental Protection Agency has set as its standard, and category 2 is the arsenic concentration in water (10–99 μg/L) that individuals who reside in the United States are currently exposed to. Categories 3 and 4 were chosen to ensure a similar number of patients in all categories.

Tumor Samples and Tissue Selection

One hematoxylin and eosin (H&E)-stained slide of each tumor section was used to confirm the grade (G1–G3) of each tumor using World Health Organization (WHO) guidelines *(13)*. The tumor stage described in the pathology report was used for Argentinean patients, because usually only one representative tumor block was available for each patient. In Chile, all existing tumor blocks were collected for each patient, and newly cut H&E-stained sections from all blocks were reviewed to confirm stage. Stage and grading of tumors was in accordance with WHO guidelines *(14)*. Medical records were not available for Chilean patients.

To obtain DNA for CGH analyses, tumor sections were microdissected to increase the concentration of tumor cells in the tissue sample to be extracted. Paraffin-embedded tumor sections (5 μm) were placed on glass slides for microdissection. Using an adjacent H&E-stained slide of the same tumor for orientation, one or two 5-μm deparaffinized, methyl green (0.1%)-stained

tumor sections were microdissected, as described previously *(15)*. Methyl green stain was used to better visualize the tumor tissue on the slide and to compare it to the adjacent H&E-stained slide from the same tumor. An area of the tumor containing a minimal amount of contamination with normal cells was selected. Areas containing necrotic tissue and/or cautery artifact were avoided. When multiple tumor pieces were available, the largest piece containing the most pure tumor cell population was selected as representative of the primary lesion. The minimum size needed for CGH analysis was 0.3 mm^2, or approximately 500 cells. The microdissected sections of tumor were then placed in microcentrifuge tubes with a volume of tissue extraction buffer containing 1× polymerase chain reaction buffer (10 m*M* Tris, 1.5 m*M* $MgCl_2$, 50 m*M* KCl), 0.5% Tween 20, and 0.4 mg/mL proteinase K. The volume of tissue extraction buffer was adjusted to contain approximately 500–1000 cells per 15 μL. DNA was isolated from the cells, and whole genome amplification was conducted as described previously *(15,16)*.

CGH

CGH was performed as described previously *(15–21)*. Briefly, for each experiment, one negative control hybridization was performed. This hybridization compared normal DNA to normal DNA labeled with different fluorochromes. A positive control hybridization comparing human breast cancer cell line (MPE) DNA to normal lymphocyte DNA was also performed. The MPE DNA sample contained several chromosomal alterations and high-level alterations that should be observed if probe labeling, the CGH hybridization, and image analysis steps were conducted under optimal conditions. Details of the probe labeling and CGH hybridization have been described previously *(15–21)* and can also be obtained from the Waldman Laboratory Web site (http://cc.ucsf.edu/people/waldman/Protocols/pcrmicrocgh.html).

Successful hybridization was demonstrated by good intensity signals, that is, with smooth, homogeneous staining over the entire metaphase spread. At least five metaphase spreads were examined for each tumor sample and each negative and positive control. DNA was detected using fluorescence microscopy methods previously described *(15–18)*. Tumor-to-normal lymphocyte DNA (reference) fluorescence intensity ratios were calculated along chromosomal arms from each metaphase spread, and their ratios were compared. If the DNA copy numbers in both the tumor and reference samples are equal, the tumor-to-reference fluorescence ratios will be the same, i.e., 1.0. The fluorescence ratio for all chromosomes in the negative control hybridization was expected to be 1.0, with small standard deviations (SDs). If tumor DNA is gained, the tumor fluorescence intensity would be greater than the reference intensity, and the ratio of tumor-to-reference fluorescence intensity would be greater than 1.0. Conversely, if tumor DNA is lost, the fluorescence intensity ratio would be less than 1.0. The fluorescence ratios computed for each chromosome in the positive control sample hybridization were examined to ensure that the known genetic alterations could be observed with the desired intensity.

In this study, gains and losses of tumor DNA were considered substantially different from normal DNA copy number if the mean and SD from the average of more than five metaphase images were more than 1.2 or less than 0.85 (i.e., the SD lines of the ratio also had to be more than 1.2 or less than 0.85). These values were defined based on experiments analyzing the hybridization of normal DNA versus normal DNA—each labeled with a different fluorochrome. Each cut point is three SDs greater than or less than the mean fluorescence ratios observed from such experiments and is based on a logarithmic scale. High-level amplifications were defined as gains of tumor DNA copy number with both a mean fluorescence intensity ratio and an SD above 1.5.

After one hybridization experiment was performed per sample, a separate hybridization experiment was conducted using the same normal and tumor DNA pair; however, this time the fluorochromes used to prepare each tumor and normal DNA probe were reversed. After analyzing the results from each hybridization independently, the results from both the CGH hybridizations performed with each sample were compared to determine the final number of alterations (gains and losses) observed in that sample. Each genetic alteration had to be present in both hybridizations to be considered as a gain or a loss. Interpretations of chromosomal alterations at 1 terminal portion of the p arm (pter), 19 pter, and 22 pter (and 4 pter and 13 pter in the opposite direction) were interpreted with caution because artifactual gains and losses on these chromosomal areas are sometimes observed at these loci. If alterations were observed at these loci, the negative and positive control samples were examined to determine if the same patterns of alterations were observed. If the alteration was present in the negative and positive control hybridizations performed for that experiment, alterations at these loci were ignored. If the alteration was not present in the negative and positive control hybridizations, each alteration was subject to the previously described scoring criteria if considered substantial.

Statistical Analysis

The overall mean number of chromosomal alterations in tumors from each arsenic exposure category was compared to that in the category with the lowest exposure (category 1), as was the number of chromosomal gains and losses per tumor. In addition to arsenic exposure, patient categories were stratified by tumor stage, tumor grade, sex, and smoking history (i.e., ever smokers versus never smokers). Changes were considered high frequency if they were present in at least 20% of all tumors (combined). Chi-square (χ^2) tests were applied to contingency (2 × 2) table analyses and were used to test for differences in alteration prevalence among sex and among smoking history categories. Each dependent variable (chromosomal gains or losses) was measured as a dichotomous variable (i.e., yes or no) per chromosome arm. More than one gain (or loss) per arm was scored as one event. Because the chromosomal gains and losses were not simple counts reflecting the actual number of changes per chromosome arm, findings were treated as ordered data rather than as counts. Therefore, ordered logistic regression analysis was used to test for trend of chromosomal alterations within tumor stage, tumor grade, and arsenic exposure categories before and after adjusting for stage and grade. Comparison of regression models with and without interaction (i.e., smoking history and arsenic exposure) was conducted with a likelihood ratio test. All analyses were conducted using STATA 6.0 software (Stata Corporation, College Station, TX). All statistical tests were two-sided.

RESULTS

Clinical Variables and Chromosomal Alterations in Bladder Tumors

The distributions of tumors in patients from Argentina and Chile by tumor stage, tumor grade, and smoking history are shown in Table 1. There were more male patients in the Argentinean group than in the Chilean group (90% versus 66%, respectively), and a higher proportion of patients from Argentina were ever smokers than that of patients from Chile (80% versus 66%, respectively).

The mean number of chromosomal alterations per tumor was assessed using CGH to determine genome copy number alterations per chromosome arm. On average, there was a mean of 6.8 ± 6.1 alterations (defined as having a mean fluorescence ratio from all metaphase images collected and an SD above 1.2 or below 0.85) per tumor (range = 0–27 changes per tumor) (Table 2). Among these changes, there was an average of 3.1 ± 3.3 gains per tumor (range = 0–15), 3.7 ± 3.2 losses per tumor (range = 0–12), and 0.2 ± 0.7 high-level amplifications per tumor (range = 0–4) (data not shown). The most frequent chromosome arm gains (those found in 20% or more of tumors) were located on chromosomes 1q (25%), 5p (22%), 8q (29%), 17q (21%), and 20q (22%). The most frequent chromosome arm losses (those found in 20% or more of tumors) were located on chromosomes 5q (24%), 8p (33%), 9p (35%), 9q (46%), 11p (25%), and 17p (24%) (data not shown). Specific chromosomal alterations for each individual patient are available online as supplemental data (available at http://jncicancerspectrum.oupjournals.org/jnci/content/vol94/issue22/index.shtml). The mean number of chromosomal alterations, gains, and losses increased with both tumor grade and tumor stage (P<.001) (Table 2). The mean number of chromosomal alterations per tumor did not differ by patients' sex or smoking history (Table 2).

Table 1. Clinical variables for all bladder cancer tumors

Variable	Argentina N = 94 (76%)	Chile N = 29 (24%)	Total N = 123 (100%)
Tumor stage*, n (%)			
Ta	27 (29)	7 (24)	34 (28)
T1	37 (39)	13 (45)	50 (41)
T2	18 (19)	9 (31)	27 (22)
T3–T4	10 (11)	0	10 (8)
Missing†	2 (2)	0	2 (2)
Tumor grade*, n (%)			
1	29 (31)	6 (21)	35 (28)
2	44 (47)	15 (52)	59 (48)
3	21 (22)	8 (28)	29 (24)
Sex‡, n (%)			
Male	85 (90)	19 (66)	104 (85)
Female	9 (10)	10 (34)	19 (15)
Smoking history, n (%)			
Males			
Ever	74 (87)	17 (89)	91 (88)
Never	11 (13)	2 (11)	13 (13)
Females			
Ever	1 (11)	2 (20)	3 (16)
Never	8 (89)	8 (80)	16 (84)
Total			
Ever	75 (80)	19 (66)	94 (76)
Never	19 (20)	10 (34)	29 (24)

*Tumor staging and grading were in accordance with World Health Organization *(13)* and American Joint Committee on Cancer *(14)* guidelines.

†Medical or pathology reports were not available for these patients.

‡P<.001, chi-square test comparing Argentinean and Chilean patients.

Table 2. Chromosomal alterations in tumors from bladder cancer patients stratified by tumor stage, tumor grade, smoking history, and sex

Variable	No. of tumor samples	No. of chromosomal alterations (mean ± SD)*	No. of chromosomal gains (mean ± SD)*	No. of chromosomal losses (mean ± SD)*
Total	123	6.8 ± 6.1	3.1 ± 3.3	3.7 ± 3.2
Tumor stage†				
Ta	34	5.1 ± 6.4	2.4 ± 3.5	2.7 ± 3.1
T1	50	5.6 ± 4.9	2.4 ± 2.7	3.2 ± 2.7
T2–T4	37	9.8 ± 6.3	4.8 ± 3.5	5.3 ± 3.4
P_{trend}‡		<.001	<.001	<.001
Tumor grade†				
1	35	2.6 ± 2.6	0.8 ± 1.2	1.8 ± 1.7
2	59	7.9 ± 6.2	3.5 ± 3.4	4.4 ± 3.3
3	29	9.5 ± 6.4	4.9 ± 3.5	4.6 ± 3.4
P_{trend}‡		<.001	<.001	<.001
Smoking history				
Ever	94	6.9 ± 6.0	3.1 ± 3.3	3.8 ± 3.1
Never	29	6.1 ± 6.4	2.9 ± 3.3	3.2 ± 3.3
P_{trend}§		.51	.73	.37
Sex				
Male	104	6.7 ± 6.1	3.0 ± 3.3	3.7 ± 3.1
Female	19	6.6 ± 6.2	2.9 ± 3.2	3.6 ± 3.2
P_{trend}§		.89	.84	.91

*Values are per tumor. SD = standard deviation.

†Tumor stage excludes two individuals with missing medical or pathology reports. Tumor staging and grading were in accordance with World Health Organization *(13)* and American Joint Committee on Cancer *(14)* guidelines.

‡P value is calculated from the ordered logistic test for trend.

§P value is calculated from the chi-square test.

Effect of Arsenic Exposure on Chromosomal Alterations

The mean number of chromosomal alterations per tumor increased with increasing 5-year peak average arsenic exposure (5.7 ± 5.1 for category 1; 5.6 ± 5.1 for category 2; 7.3 ± 7.4 for category 3, and 9.1 ± 6.5 for category 4; P_{trend} = .03; Table 3). Stronger trends were observed after adjusting for tumor stage and grade. The association between chromosomal alterations,

Table 3. Chromosomal alterations in tumors from bladder cancer patients stratified by arsenic exposure category

Exposure category*	No. of tumors	No. of chromosomal alterations (mean ± SD)†	No. of chromosomal gains (mean ± SD)†	No. of chromosomal losses (mean ± SD)†
1	45	5.7 ± 5.1	2.4 ± 2.7	3.3 ± 2.9
2	24	5.6 ± 5.1	2.4 ± 2.9	3.2 ± 2.8
3	29	7.3 ± 7.4	3.8 ± 4.1	3.6 ± 3.4
4	25	9.1 ± 6.5	4.1 ± 4.0	5.1 ± 3.4
P_{trend}‡		.03	.03	.06
P_{trend} (adjusted)§		.02	.008	.05

*Average 5-year peak concentration (with proxy arsenic concentrations from nearest well of similar depth when necessary): category 1 = 0–<10 μg/L, category 2 = 10–99 μg/L, category 3 = 100–299 μg/L, category 4 = ⩾300 μg/L. SD = standard deviation.

†Values are per tumor; alterations are the sum of gains and losses on all chromosome arms.

‡P value is calculated from the ordered logistic test for trend.

§P value for the test for trend, adjusted for tumor stage and grade.

tumor grade, tumor stage, and arsenic exposure are presented in Table 4. The mean number of chromosomal alterations increased among arsenic exposure categories in G2 and G3 tumors (all stages) but not in G1 tumors (all stages). In exposure category 1, G2–G3 tumors (all stages) contained 6.3 ± 5.5 alterations per tumor, whereas G2–G3 tumors in category 4 (highest exposure) contained 10.5 ± 6.4 alterations per tumor, a 1.7-fold difference (P_{trend} = .01). This difference by arsenic exposure category was most dramatic in Ta/G2–G3 tumors. Exposure category 1 Ta/G2–G3 tumors contained only 3.6 ± 3.5 alterations per tumor, whereas exposure category 4 Ta/G2–G3 tumors contained 13.3 ± 11.0 alterations per tumor, a 3.7-fold difference (P_{trend} = .07). By contrast, G1 tumors of any stage showed little difference in alterations by arsenic exposure.

We also examined the relationship between number of chromosomal alterations and arsenic exposure after stratification by smoking history to determine if there was evidence of an interaction between these two bladder carcinogens (Table 5). In the 94 ever smokers, the average total number of chromosomal alterations increased statistically significantly with increasing arsenic exposure after adjusting for tumor stage and grade (P_{trend} = .03). In never smokers, there was a similar trend; however, the difference in the number of chromosomal losses was not statistically significant, possibly because of the small number of bladder cancer patients who never smoked (n = 29). Comparison of the results from regression model analyses conducted with and without interaction (smoking history and arsenic exposure) was conducted using a likelihood ratio test. Ever smoking and arsenic exposure combined did not cause an increase in the total number of chromosomal alterations, gains, or losses than arsenic exposure alone (data not shown).

Specific Chromosomal Alterations

Specific types of high-frequency chromosomal alterations (restricted to changes seen in ⩾20% of tumors in at least one of the comparison groups) were tested for association with tumor stage, tumor grade, and smoking history (Table 6). Because of the large number of comparisons, the results for tests of trend with *P* values greater than .001 should be interpreted with caution. Except for losses on chromosomes 5q and Y, the alterations associated with tumor stage were a subset of the changes that were also associated with tumor grade. Two of three specific chromosomal alterations associated with ever smoking were also associated with tumor stage and grade (except the loss of chromosome 9q). Interestingly, chromosome 9q loss was found more frequently in the 29 never smokers than in the 94 ever smokers (62% versus 42%, respectively; *P* = .05) (data not shown).

P values for tests of trend of high-frequency chromosomal alterations associated with arsenic exposure are also shown in Table 6. Chromosomes 3q and 11q gains were seen at higher frequencies with increasing arsenic exposure (P_{trend} = .03, adjusted for tumor stage and tumor grade, for both chromosomes). Regions of loss that increased in frequency with exposure were located on chromosome 8p (P_{trend} = .05), 17p (P_{trend}<.001), and 9q (P_{trend} = .03) (all adjusted for tumor stage and grade). The specific chromosomal changes associated with increasing arsenic exposure were similar to those associated with tumor stage and/or grade except 9q loss, which was negatively associated with smoking history.

Discussion

In this study, CGH was used to characterize chromosomal alterations in bladder tumors from patients exposed to arsenic and tobacco smoke. We found that the total number of chromosomal alterations was higher in people exposed to higher levels of arsenic in their drinking water. This finding raises the possibility that bladder tumors from patients who have been exposed to high levels of arsenic (>300–600 μg/L) are less genetically stable than tumors from patients exposed to low levels of arsenic (<10 μg/L) in their drinking water. The trend was stronger in G2–G3 tumors than in G1 tumors. The mean number of alterations also increased with tumor stage and tumor grade independently of arsenic exposure but was not associated with smoking history.

CGH has been used previously to characterize carcinomas of the bladder in European *(21,22)* and American *(23)* cohorts and bladder cancers associated with schistosome infection *(24)*. Earlier studies of the use of CGH in characterizing chromosomal alterations in bladder cancer did not consider the role of environmental exposures in their analyses, nor did they attempt to

Table 4. Mean number of chromosomal alterations in tumors from bladder cancer patients stratified by arsenic exposure category and tumor stage and grade

		Exposure category								
		1		2		3		4		
Tumor grade	Tumor stage	No. of tumors	No. of chromosomal alterations (mean ± SD)‡	No. of tumors	No. of chromosomal alterations (mean ± SD)‡	No. of tumors	No. of chromosomal alterations (mean ± SD)‡	No. of tumors	No. of chromosomal alterations (mean ± SD)‡	P_{trend}†
1	Ta	3	3.3 ± 4.9	5	1.2 ± 1.1	4	3.3 ± 3.3	3	3.0 ± 3.6	.67
	T1	7	3.6 ± 2.5	3	0.7 ± 1.2	7	2.1 ± 2.1	1	2.0	.42
	T2–4	0	—	1	2.0	0	—	1	7.0	
	Total	10	3.5 ± 3.1	9	1.1 ± 1.1	11	2.5 ± 2.5	5	3.6 ± 3.2	.79
2–3	Ta	9	3.6 ± 3.5	3	9.0 ± 2.7	4	9.3 ± 11.9	3	13.3 ± 11.0	.07
	T1	12	7.0 ± 5.1	7	6.6 ± 5.0	6	5.7 ± 3.6	7	9.7 ± 6.3	.42
	T2–4	14	7.6 ± 6.4	5	9.8 ± 5.4	8	14.3 ± 6.5	10	10.1 ± 5.5	.11
	Total	35	6.3 ± 5.5	15	8.3 ± 4.7	18	10.3 ± 7.8	20	10.5 ± 6.4	.01

*Average 5-year peak concentration (with proxy arsenic concentrations from nearest well of similar depth when necessary): category 1 = 0–<10 μg/L, category 2 = 10–99 μg/L, category 3 = 100–299 μg/L, category 4 = ⩾300 μg/L. Tumor staging and grading were in accordance with World Health Organization *(13)* and American Joint Committee on Cancer *(14)* guidelines. SD = standard deviation.

†*P* value is calculated from the ordered logistic test for trend.

‡Alterations are the sum of gains and losses on all chromosome arms.

Table 5. Chromosomal alterations in tumors from bladder cancer patients stratified by arsenic exposure category and smoking history

	Ever smokers (N = 94)				Never smokers (N = 29)			
Exposure category*	No. of tumors	No. of chromosomal alterations (mean ± SD)†	No. of chromosomal gains (mean ± SD)†	No. of chromosomal losses (mean ± SD)†	No. of tumors	No. of chromosomal alterations (mean ± SD)†	No. of chromosomal gains (mean ± SD)†	No. of chromosomal losses (mean ± SD)†
1	34	6.3 ± 5.3	2.7 ± 2.8	3.6 ± 3.0	11	3.8 ± 4.1	1.6 ± 2.1	2.4 ± 2.3
2	20	5.7 ± 5.2	2.4 ± 3.0	3.3 ± 2.8	4	5.3 ± 5.3	2.5 ± 2.6	2.8 ± 2.8
3	25	7.1 ± 7.1	3.6 ± 4.1	3.5 ± 3.2	4	8.9 ± 9.8	4.8 ± 4.7	4.0 ± 5.4
4	15	9.9 ± 6.0	4.3 ± 3.2	5.7 ± 3.2	10	7.9 ± 7.3	3.8 ± 5.8	4.1 ± 3.7
P_{trend}‡		.11	.16	.10		.08	.04	.25
P_{trend} (adjusted)§		.03	.02	.06		.15	.07	.52

*Average 5-year peak concentration (with proxy arsenic concentrations from nearest well of similar depth when necessary): category 1 = 0–<10 μg/L, category 2 = 10–99 μg/L, category 3 = 100–299 μg/L, category 4 = ⩾300 μg/L. SD = standard deviation.

†Alterations are the sum of gains and losses on all chromosome arms.

‡*P* value is calculated from the ordered logistic test for trend.

§*P* value for the test for trend, adjusted for tumor stage and grade.

Table 6. *P* values for tests of trend in specific chromosomal alterations in tumors from bladder cancer patients stratified by tumor stage, tumor grade, smoking history, and arsenic exposure*

Chromosomal alterations†	Tumor stage	Tumor grade	Smoking history‡	Arsenic exposure
Gains				
3q	.04	.01	—§	.04 (.03)‖
5p	.04	.003	—	—
6p	.03	.002	—	—
7p	—	.04	—	—
10p	—	.01	—	—
8q	.02	.001	.05 (.04)‖	—
11q	.006	.02	—	.04 (.03)‖
17q	.004	.006	—	—
19q	—	.05	—	—
20q	—	.03	—	—
Losses				
8p	.001	.001	.05 (.07)‖	.07 (.05)‖
17p	—	.007	—	.001 (.001)‖
5q	.002	.02	—	—
9q	—	—	.06 (.03)‖	.03 (.03)‖
18q	.03	.05	—	—
Y	.03	—	—	—

**P* values were calculated using the ordered logistic test for trend. All trends increased with tumor stage, tumor grade, smoking history, and arsenic exposure except for chromosome 9q loss, which was inversely associated with smoking history. Tumor staging and grading were in accordance with World Health Organization *(13)* and American Joint Committee on Cancer *(14)* guidelines.

†Chromosomal alterations reported were observed in ⩾20% of tumors in at least one comparison group.

‡Smoking history is defined as ever smoked and never smoked.

§— = *P*>.1 for test for trend.

‖Values in parentheses are adjusted for tumor stage (Ta, T1, T2–T4) and tumor grade (G1–G3).

deal with possible confounding factors other than descriptively, with tumor stage and grade. In this study, by contrast, we obtained detailed lifestyle information so that we could determine whether tumors from patients exposed to one or both of two known bladder carcinogens, arsenic and tobacco smoke, differed in either the number or type of chromosomal alterations as assessed by CGH.

The mean number of alterations found in previous studies of high-stage (T2–4) bladder cancer (8–11 alterations per tumor) *(21,22,25)* is similar to the number of alterations found in our study, 6.8 ± 6.1 alterations per tumor (range = 0–27). Moreover, the types of chromosomal alterations found in previous studies *(21–28)* are also similar to those that we found. For example, frequently reported chromosomal gains described in the above studies are found on chromosomes 7p, 8q, and 11q, and chromosome losses are frequently observed on chromosomes 8 and 9.

The current study demonstrates that the number of chromosomal alterations in bladder cancer increased with increasing arsenic exposure. This trend remained after stratification by smoking history and was not stronger in ever smokers than it was in never smokers. Among individuals with high-grade tumors (G2–G3), those who had been exposed to high levels (>300–600 μg/L) of arsenic in their lifetime had more chromosomal alterations than those exposed to low levels (<10 μg/L). This effect was most pronounced in high-grade superficial (Ta/G2–G3) tumors, with chromosomal alterations of 3.6 ± 3.5 per tumor in exposure category 1 and 13.3 ± 11.0 alterations per tumor in exposure category 4 (P_{trend} = .07).

The number of alterations that we found in the high-arsenic exposure category of tumors was much greater than that found in a previous CGH study of noninvasive (Ta) bladder tumors. Zhao et al. *(29)* found an average of 1.9 ± 2.0 alterations per tumor in Ta/G1, 3.1 ± 2.9 alterations per tumor in Ta/G2, and 7.7 ± 4.5 alterations per tumor in Ta/G3 tumors. In our study, high-exposure Ta/G2–G3 tumors had at least twice as many alterations per tumor as the number of alterations reported for Ta/G2–G3 tumors previously *(29)*. To explain this excess of chromosomal alterations in patients exposed to arsenic in our study, we hypothesize that the increasing overall number of chromosomal alterations observed with increasing arsenic exposure reflects the genetic instability caused by exposure. Such instability may contribute an aggressive component to these tumors from arsenic-exposed individuals, because the majority of chromosomal alterations associated with arsenic exposure are also associated with the stage (depth of invasion) and grade (tumor cell differentiation) of these tumors.

Future studies should focus on the prognosis or outcome of arsenic-exposed and unexposed bladder cancer patients to determine if mortality is greater in exposed patients and/or if tumors in arsenic-exposed patients behave more aggressively than tumors in unexposed patients. The effect of arsenic exposure on

bladder cancer incidence in the general population is not yet known; however, a recent study in Taiwan *(7),* using the arsenic concentration in well water and duration of drinking well water as an index of exposure, reported increased bladder cancer incidence with exposures as low as 10.1–50.0 μg/L.

Chromosomal alterations that were the most strongly associated with arsenic exposure were gains on chromosomes 3q and 11q and losses on chromosomes 8p, 17p, and 9q. Gains on 3q were also associated with tumor stage and grade. The association of arsenic exposure with gains on 11q is interesting, because chromosome locus 11q13 is the site of cyclin D1 and INT2 genes and has been commonly reported as being amplified in bladder cancer *(30,31).* We found chromosomal gains near that particular locus (i.e., 11q13). We also found that 11q gains were associated with tumor grade and stage. Higher resolution analysis of chromosome regions, such as by CGH microarray or fluorescence *in situ* hybridization, would be necessary to demonstrate gene-specific alterations.

In this study, deletion of part or all of chromosome 9q was increased in tumors from patients exposed to high levels of arsenic compared with tumors from patients exposed to low levels of arsenic. Moreover, a positive trend of 9q loss was associated with increasing arsenic exposure. Chromosome 9 is frequently lost in bladder cancer, suggesting that a bladder cancer suppressor gene may be found on this chromosome. Although the bladder cancer-associated gene on the q arm of chromosome 9 is still not known, a number of candidate genes exist, such as the putative tumor suppressor gene (PTCH) located at 9q22. Identification of tumor suppressor genes on chromosome 9 is difficult because high proportions of bladder tumors have lost all of chromosome 9, leaving few cases with enough genetic material for an informative study *(32,33).* In our study, 9q losses were not associated with tumor stage or grade and were inversely associated with smoking history, possibly because these changes are acquired early in most bladder cancer tumors and may not be specific to tumors from arsenic-exposed patients *(34).*

The strongest trends were observed when comparing tumors from patients exposed to high levels of arsenic (>300–600 μg/L) with those from patients exposed to low levels of arsenic (<10 μg/L), involving deletion of part or all of chromosome arm 17p (P_{trend}<.001). This finding suggests that 17p loss may play a role in arsenic-induced bladder tumorigenesis. Moreover, 17p loss was associated only with arsenic exposure and tumor grade and not with stage or smoking history. The most likely candidate gene target associated with 17p loss or deletion is p53. For example, one small study of bladder tumors suggested that arsenic exposure might be related to a mutational hotspot at codon 175 of p53 *(35).* However, studies *(36)* of skin tissue from patients with arsenic-related Bowen's disease found no detectable p53 mutations such as those found in UV-related skin cancers. Other studies *(37,38)* have suggested that more than one relevant tumor suppressor gene may be present on the p arm of chromosome 17.

Although this study demonstrates that tumors from arsenic-exposed patients are less genetically stable than those from unexposed patients, the mechanism of this instability is still unknown. Mechanisms that may play a role in this genetic instability include interaction between arsenic and proteins involved in cell cycle pathways, such as p53, as previously described; inhibition of DNA repair enzymes that mediate the genotoxic and mutagenic effects of arsenic and other chemicals; and aberrant DNA methylation. Li and Rossman *(39)* and Lee-Chen et al. *(40)* have shown that sodium arsenite inhibited the activity of DNA ligase I and II. Other studies have shown that arsenic alters the types of mutations observed after exposure to UV radiation *(41,42).* This finding suggests that arsenic may interfere with early or late steps in DNA repair. Arsenic has also been shown to increase activities of DNA polymerase beta, O^6-methylguanine-DNA methyltransferase, and DNA ligase I, II, and III at various doses and in different cell models *(43).* Moreover, arsenic is chemically reactive and binds to sulfhydryl groups in proteins *(3).* Arsenic has been shown to block DNA binding at the glucocorticoid receptor through interactions with the DNA-binding domain of proteins *(44).* Finally, many DNA repair enzymes contain vicinal dithiols, such as UV radiation resistance-associated (UVRA) protein *(45),* poly adenosine diphosphate-ribose protein (PARP) *(46,47),* post replication repair protein 18p (RAD-18) *(48),* and xeroderma pigmentosum complementation group A protein-I (XPAC) *(49),* which could make them susceptible to inhibition by arsenic.

Methylation changes of genes or their control regions could result in altered gene expression and carcinogenesis *(50,51).* Recently, Mass and Wang *(52)* demonstrated that exposure of human lung adenocarcinoma A549 cells to sodium arsenite produced dose-responsive hypermethylation within a 341-base-pair fragment of the p53 promoter, which decreases its transcriptional activity.

Another *a priori* hypothesis of this study was that arsenic may cause an increase in gene amplifications in tumors from arsenic-exposed individuals. Lee et al. *(53)* have shown that arsenic caused amplification of the dihydrofolate reductase gene *in vitro.* The results of our study, however, do not support this hypothesis, because high-level gene amplifications were rare overall and occurred at similar frequencies in tumors from arsenic-exposed and unexposed patients. Our study showed that bladder tumors from patients who have been exposed to arsenic had more chromosomal changes and, thus, were less genetically stable than those from patients who were not exposed to arsenic. *In vivo* and *in vitro* studies of rodents and humans have reported various forms of chromosomal alterations, including the induction of micronuclei *(2,54–63).* Arsenic-induced chromosomal alterations have also been demonstrated *in vivo* and *in vitro* in human lymphocytes and in exfoliated bladder cells from exposed individuals *(59–63).*

Bladder cancer is also associated with smoking *(64).* However, when we compared the frequency and types of chromosomal alterations in bladder tumors from ever smokers versus never smokers by CGH, no difference in the frequency of chromosome arm alterations in bladder tumors was observed; however, there were differences in the specific locations of changes within the genome. These differences included gains on chromosome arm 8q and losses on chromosome arms 8p and 9q in ever smokers. Additional chromosomal alterations may have occurred that cannot be detected with CGH because tobacco smoke, a complex mixture of carcinogens, could cause smaller genetic changes in urothelial cell DNA. Such small alterations could be detected only with more sensitive methods, such as loss of heterozygosity (LOH) or CGH microarrays. It is noteworthy that only chromosome 9q loss was more frequent in never smokers than it was in ever smokers. These findings are in contrast to a recent LOH analysis of bladder tumor DNA by Zhang et al.

(65), which suggested that regional chromosome 9 loss may be involved in smoking-related bladder carcinogenesis. The differences between our findings and those of Zhang et al. may reflect the lower sensitivity of CGH to detect small genetic alterations compared with those that can be identified with LOH.

In conclusion, we have shown that increasing arsenic exposure is associated with increases in both the frequency and specific types of genetic alterations in bladder tumors. Although the exact molecular mechanism of the carcinogenicity of arsenic is not yet known, we hypothesize that arsenic may cause increased genetic instability in bladder tumors, possibly by deregulating cell cycle control pathways via epigenetic mechanisms or by reducing the ability of the cell to respond properly to or to repair DNA damage. Both mechanisms could enhance the rate of bladder cancer development, chromosomal alterations, and tumor progression. Furthermore, most of the specific chromosomal alterations that were associated with increasing arsenic exposure in our study were also associated with tumor stage and tumor grade, suggesting that bladder tumors from arsenic-exposed individuals may behave more aggressively and may result in increased mortality.

REFERENCES

(1) International Agency for Research on Cancer (IARC). IARC monographs on the evaluation of the carcinogenic risk to man: some metals and metallic compounds. Lyon (France): IARC; 1980. p. 39–141.

(2) National Research Council (NRC). Arsenic in drinking water. Washington (DC): National Academy Press; 1999. p. 83–101.

(3) Chen CJ, Chuang YC, Lin TM, Wu HY. Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: high-arsenic artesian well water and cancers. Cancer Res 1985;45:5895–9.

(4) Chen CJ, Chuang YC, You SL, Lin HY. A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan. Br J Cancer 1986;53:399–405.

(5) Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH, Wu HY. Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 1988;8:452–60.

(6) Chen CJ, Chen CW, Wu MM, Kuo TL. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer 1992;66:888–92.

(7) Chiou HY, Chiou ST, Hsu YH, Chou YL, Tseng CH, Wei ML, et al. Incidence of transitional cell carcinoma and arsenic in drinking water: a follow-up study of 8,102 residents in an arseniasis-endemic area in northeastern Taiwan. Am J Epidemiol 2001;153:411–8.

(8) Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, et al. Cancer risks from arsenic in drinking water. Environ Health Perspect 1992;97:259–67.

(9) Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 1998;147:660–9.

(10) Hopenhayn-Rich C, Biggs ML, Fuchs A, Bergoglio R, Tello E, Nicolli H, et al. Bladder cancer mortality associated with arsenic in drinking water in Argentina. Epidemiology 1996;7:117–24.

(11) Ferreccio C, González C, Milosavjlevic V, Marshall G, Sancha AM, Smith AH. Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology 2000;11:673–9.

(12) Kalman DA. Quantitation of arsenic species in urine for exposure assessment studies. J Res Natl Bur Stand 1988;93:315–7.

(13) Mostofi FK. International histologic classification of tumors. A report by the Executive Committee of the International Council of Societies of Pathology. Cancer 1974;33:1480–4.

(14) American Joint Committee on Cancer (AJCC). Urinary bladder: AJCC cancer staging manual. 5th ed. Philadelphia (PA): Lippincott-Raven; 1997.

(15) Waldman FM, DeVries S, Chew KL, Moore DH, Kerlikowske K, Ljung BM. Chromosomal alterations in ductal carcinomas in situ and their in situ recurrences. J Natl Cancer Inst 2000;92:313–20.

(16) Willenbucher RF, Zelman SJ, Ferrell LD, Moore DH, Waldman FM. Chromosomal alterations in ulcerative colitis-related neoplastic progression. Gastroenterology 1997;113:791–801.

(17) Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–21.

(18) Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, et al. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994;10:231–43.

(19) Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, et al. Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci U S A 1994; 91:2156–60.

(20) Isola JJ, Kallioniemi OP, Chu LW, Fuqua SA, Hilsenbeck SG, Osborne CK, et al. Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 1995; 147:905–11.

(21) Kallioniemi A, Kallioniemi OP, Citro G, Sauter G, Devries S, Kerschmann R, et al. Identification of gains and losses of DNA sequences in primary bladder cancer by comparative genomic hybridization. Genes Chromosomes Cancer 1995;12:213–9.

(22) Richter J, Beffa L, Wagner U, Schraml P, Gasser TC, Moch H, et al. Patterns of chromosomal imbalances in advanced urinary bladder cancer detected by comparative genomic hybridization. Am J Pathol 1998;153:1615–21.

(23) Hovey RM, Chu L, Balazs M, DeVries S, Moore D, Sauter G, et al. Genetic alterations in primary bladder cancers and their metastases. Cancer Res 1998;58:3555–60.

(24) Muscheck M, Abol-Enein H, Chew K, Moore D 2nd, Bhargava V, Ghoneim MA, et al. Comparison of genetic changes in schistosome-related transitional and squamous bladder cancers using comparative genomic hybridization. Carcinogenesis 2000;21:1721–6.

(25) Koo SH, Kwon KC, Ihm CH, Jeon YM, Park JW, Sul CK. Detection of genetic alterations in bladder tumors by comparative genomic hybridization and cytogenetic analysis. Cancer Genet Cytogenet 1999;110:87–93.

(26) Richter J, Jiang F, Görög JP, Sartorius G, Egenter C, Gasser TC, et al. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. Cancer Res 1997;57:2860–4.

(27) Richter J. Evolution of schistosomiasis-induced pathology after therapy and interruption of exposure to schistosomes: a review of ultrasonographic studies. Acta Trop 2000;77:111–31.

(28) Voorter C, Joos S, Bringuier PP, Vallinga M, Poddighe P, Schalken J, et al. Detection of chromosomal imbalances in transitional cell carcinoma of the bladder by comparative genomic hybridization. Am J Pathol 1995;146: 1341–54.

(29) Zhao J, Richter J, Wagner U, Roth B, Schrami P, Zellweger T, et al. Chromosomal imbalances in noninvasive papillary bladder neoplasms (pTa). Cancer Res 1999;59:4658–61.

(30) Proctor AJ, Coombs LM, Cairns JP, Knowles MA. Amplification at chromosome 11q13 in transitional cell tumours of the bladder. Oncogene 1991; 6:789–95.

(31) Shaw ME, Knowles MA. Deletion mapping of chromosome 11 in carcinoma of the bladder. Genes Chromosomes Cancer 1995;13:1–8.

(32) Tsai YC, Nichols PW, Hiti AL, Williams Z, Skinner DG, Jones PA. Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res 1990;50:44–7.

(33) Simoneau AR, Jones PA. Bladder cancer: the molecular progression to invasive disease. World J Urol 1994;12:89–95.

(34) Spruck CH, Ohneseit PF, Gonzalez-Zulueta M, Esrig D, Miyao N, Tsai YC, et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994;54:784–8.

(35) Shibata A, Ohneseit PF, Tsai YC, Spruck CH 3rd, Nichols PW, Chiang HS, et al. Mutational spectrum in the p53 gene in bladder tumors from the endemic area of black foot disease in Taiwan. Carcinogenesis 1994;15:1085–7.

(36) Hsieh LL, Chen HJ, Hsieh JT, Jee SH, Chen GS, Chen CJ. Arsenic-related Bowens's disease and paraquat-related skin cancerous lesions show no detectable ras and p53 gene alterations. Cancer Lett 1994;86:59–65.

(37) Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG. Allelotype of human bladder cancer. Cancer Res 1994;54:531–8.

(38) Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, et al. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. Nat Med 1995;1:570–7.
(39) Li J, Rossman TG. Inhibition of DNA ligase activity by arsenite: a possible mechanism of its comutagenesis. Mol Toxicol 1989;2:1–9.
(40) Lee-Chen SF, Yu CT, Wu DR, Jan KY. Differential effects of luminol, nickel, and arsenite on the rejoining of ultraviolet light and alkylation-induced DNA breaks. Environ Mol Mutagen 1994;23:116–20.
(41) Yang HL, Tu SC, Lu FJ, Chiu HC. Plasma protein C activity is enhanced by arsenic but inhibited by fluorescent humic acid associated with black-foot disease. Am J Hematol 1994;46:264–9.
(42) Wiencke JK, Yager JW, Varkonyi A, Hultner M, Lutze LH. Study of arsenic mutagenesis using the plasmid shuttle vector pZ189 propagated in DNA repair proficient human cells. Mutat Res 1997;386:335–44.
(43) Hu Y, Su L, Snow ET. Arsenic toxicity is enzyme specific and its affects on ligation are not caused by the direct inhibition of DNA repair enzymes. Mutat Res 1998;408:203–18.
(44) Simons SS Jr, Chakraborti PK, Cavanaugh AH. Arsenite and cadmium(II) as probes of glucocorticoid receptor structure and function. J Biol Chem 1990;265:1938–45.
(45) Husain I, van Houten B, Thomas DC, Sancar A. Sequences of Escherichia coli uvrA gene and protein reveal two potential ATP binding sites. J Biol Chem 1986;261:4895–901.
(46) Cherney BW, McBride OW, Chen DF, Alkhatib H, Bhatia K, Hensley P, et al. cDNA sequence, protein structure, and chromosomal location of the human gene for poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A 1987;84:8370–4.
(47) Uchida K, Morita T, Sato T, Ogura T, Yamashita R, Noguchi S, et al. Nucleotide sequence of a full-length cDNA for human fibroblast poly (ADP-ribose) polymerase. Biochem Biophys Res Commun 1987;148: 617–22.
(48) Jones JS, Weber S, Prakash L. The Saccharomyces cerevisiae RAD18 gene involves a protein that contains potential zinc finger domains for nucleic acid binding and a putative nucleotide binding sequence. Nucleic Acids Res 1988;16:7119–31.
(49) Tanaka T. Arsenic in the natural environment. Arsenic concentrations in thermal waters from Japan. Appl Organometal Chem 1990;4:197–203.
(50) Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 1998;72: 141–96.
(51) Costa M. Model for the epigenetic mechanism of action of nongenotoxic carcinogens. Am J Clin Nutr 1995;61:666S-9S.
(52) Mass MJ, Wang L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutat Res 1997;386:263–7.
(53) Lee TC, Tanaka N, Lamb PW, Gilmer TM, Barrett JC. Induction of gene amplification by arsenic. Science 1988;241:79–81.
(54) Dulout FN, Grillo CA, Seoane AI, Maderna CR, Nilsson R, Vahter M, et al. Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from northwestern Argentina exposed to arsenic in drinking water. Mutat Res 1996;370:151–8.
(55) Gonsebatt ME, Vega L, Salazar AM, Montero R, Guzman P, Blas J, et al. Cytogenetic effects in human exposure to arsenic. Mutat Res 1997;386: 219–28.
(56) International Agency for Research on Cancer (IARC). IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluation of carcinogenicity: an updating of IARC monographs volumes 1 to 42, Supplement 7. Lyon (France): IARC; 1987. p. 100–6.
(57) Jha AN, Noditi M, Nilsson R, Natarajan AT. Genotoxic effects of sodium arsenite on human cells. Mutat Res 1992;284:215–21.
(58) Larramendy ML, Popescu NC, DePaolo JA. Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberrations in human and syrian hamster cell strains. Environ Mutagen 1981;3:597–606.
(59) Moore LE, Warner ML, Smith AH, Kalman D, Smith MT. Use of the fluorescent micronucleus assay to detect the genotoxic effects of radiation and arsenic exposure in exfoliated human epithelial cells. Environ Mol Mutagen 1996;27:176–84.
(60) Moore LE, Smith AH, Hopenhayn-Rich C, Biggs ML, Kalman DA, Smith MT. Decrease in bladder cell micronucleus prevalence after intervention to lower the concentration of arsenic in drinking water. Cancer Epidemiol Biomarkers Prev 1997;6:1051–6.
(61) Moore LE, Smith AH, Hopenhayn-Rich C, Biggs ML, Kalman DA, Smith MT. Micronuclei in exfoliated bladder cells among individuals chronically exposed to arsenic in drinking water. Cancer Epidemiol Biomarkers Prev 1997;6:31–6.
(62) Warner ML, Moore LE, Smith MT, Kalman DA, Fanning E, Smith AH. Increased micronuclei in exfoliated bladder cells of individuals who chronically ingest arsenic-contaminated water in Nevada. Cancer Epidemiol Biomarkers Prev 1994;3:583–90.
(63) Vega L, Gonsebatt ME, Ostrosky-Wegman P. Aneugenic effect of sodium arsenite on human lymphocytes in vitro: an individual susceptibility effect detected. Mutat Res 1995;334:365–73.
(64) Silverman DT, Hartge P, Morrison AS, Devesa SS. Epidemiology of bladder cancer. Hematol Oncol Clin North Am 1992;6:1–30.
(65) Zhang ZF, Shu XM, Cordon-Cardo C, Orlow I, Lu ML, Millon TV, et al. Cigarette smoking and chromosome 9 alterations in bladder cancer. Cancer Epidemiol Biomarkers Prev 1997;6:321–6.

NOTES

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