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Arsenic Methylation Capability, Heme Oxygenase-1 and NADPH Quinone Oxidoreductase-1 Genetic Polymorphisms and the Stage and Grade of Urothelial Carcinomas

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Key Words
Bladder neoplasms · Arsenic · Methylation

Abstract
Background: Arsenic exposure is associated with an increased risk of urothelial carcinoma (UC). To explore the distribution of the arsenic methylation capability in patients with different stages and grades of UCs, 100 UC cases were recruited between September 2002 and May 2004 for this study. ***Methods:*** Urinary arsenic species, including inorganic arsenic (As^{III} + As^{V}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), were determined with a high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. Determining the percentages of various arsenic species among the total urinary arsenic amount assessed the arsenic methylation capability. The primary methylation index (PMI) was defined as the ratio between MMA and inorganic arsenic. The secondary methylation index (SMI) was determined as the ratio between DMA and MMA. ***Results:*** Differential effects of the arsenic methylation capability were found among patients with different stages of UCs; however, none was found among different grades. ***Conclusion:*** A significantly different distribution of the HO-1 genotype was found in subjects with different-stage UCs; however, it was not related to the NAD(P)H:quinone oxidoreductase 1 genotype.

Introduction

Urothelial carcinoma (UC) arises exclusively from the urothelium including that of the renal pelvis, ureter, bladder, and urethra, with bladder cancer being the most common form. The incidence rates of bladder UC have progressively been increasing in the past decades in Taiwan, with age-specific rates for males and females in 2000 of 10.2 and 4.4 per 10^5, respectively [1]. A study reported about a 20% incidence of upper urinary tract UC in southern Taiwan, significantly higher than rates reported worldwide [2]. One possible cause of such an unusually high incidence of UC in Taiwan has been suggested to be the consumption of arsenic-contaminated artesian well

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water. Other studies also demonstrated high incidences of UC from an arseniasis hyperendemic area of Taiwan [3–5] or non-arseniasis hyperendemic area [6], and later studies suggested that a young age, female gender, high T stage, and elevated pretreatment serum lactate dehydrogenase and creatinine levels were significantly associated with UC. Several biologic and molecular parameters have been considered as potential prognostic markers for UC, but to the present, tumor grade and stage have been the most important prognostic variables [7, 8].

In drinking water, arsenic is usually found in the form of arsenate (As^V) or arsenite (As^{III}) [9]. Inorganic arsenic is biotransformed in humans to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA); and methylation of inorganic arsenic is considered a detoxification mechanism because DMA has a relatively low toxicity and is rapidly excreted in the urine [10]. Our previous studies evaluating the inorganic arsenic methylation capability found it to be related to a risk of skin cancer [11] and bladder cancer [12]. Determining whether the arsenic methylation capability influences the prognosis of UC requires further investigation.

Apart from environmental factors, the different incidences of UC may imply polymorphism of UC-associated genes. NAD(P)H:quinone oxidoreductase 1 (NQO1), an important flavoprotein that catalyzes the two-electron reduction of quinoid compounds to hydroquinones [13], is readily conjugated to other molecules which are subsequently more readily excreted, thus protecting cells from oxidative damage [14]. The *NQO1* gene is located on chromosome 16q22. The polymorphic variant is a C-to-T point mutation at position 609 of exon 6 of *NQO1* cDNA that encodes a proline-to-serine substitution at position 187 in the amino acid sequence of the protein [15]. *NQO1* polymorphism can modify the risk of colorectal cancer [16], lung cancer [17], and bladder cancer [18]. Heme oxygenase (HO) is the rate-limiting enzyme in heme degradation; it cleaves the α-meso carbon bridge of heme, yielding equimolar quantities of carbon monoxide, biliverdin, and free iron [19]. HO-1 is a stress-response protein that can be induced by various oxidative agents, including heavy metals, inflammatory mediators, ultraviolet radiation, endotoxin, and heme/hemoglobin [20, 21]. The human HO-1 gene has been localized to the human chromosome, 22q12 [22]; a $(GT)_n$ dinucleotide repeat in the 5′ flanking region of the human HO-1 gene shows length polymorphism and can modulate the levels of gene transcription [23]. The genetic polymorphism allows study of the possible involvement of HO-1 in certain human diseases. It is hypothesized that the stage and grade of UC can be influenced by polymorphisms in the genes and by the arsenic methylation capability that modulates oxidative stress particularly by interactions with exposure to relevant environmental chemicals. We therefore recruited hospital-based UC patients in order to investigate the distribution of *NQO1* and *HO-1* genetic polymorphisms and the arsenic methylation capability on the stage and grade of UC.

Materials and Methods

Study Subjects and Questionnaire Interview

One hundred patients with pathologically proven UC (with an age range of 24–93 years) were recruited from the Department of Urology, Chi-Mei Medical Center, between September 2002 and May 2004. Almost all UC cases came from Tainan City or near arsenic-contaminated areas of southwestern Taiwan. A tap water supply system was implemented in the arsenic-contaminated areas of southwestern Taiwan in the early 1960s, but its coverage remained low until the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. Bladder cancer was staged into three groups: superficial (T_a, T_1, and T_{is}), locally advanced (T_2–4N0M0), and metastatic (N+ or M+). Tumor grading was based on the WHO 1999 classification system.

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. Information collected included demographic and socioeconomic characteristics, general potential risk factors for malignancies such as lifestyle, alcohol consumption, cigarette smoking in quantified details, exposure to potential occupational and environmental carcinogens such as hair dyes and pesticides, chronic medication history, consumption of conventional and alternative medicines, and personal and family history of urological diseases. Study subjects who provided informed consent were administered the questionnaire interview; urine and blood samples were then collected on-site and urine samples were stored at –20°C, while blood samples were separated into plasma and buffy coat fractions and then stored at –80°C until further use for urinary arsenic speciation and the gene polymorphism assay, respectively.

Determination of Urinary Arsenic Species

It has been shown that urinary arsenic species are stable for at least 6 months when preserved at –20°C [24]; thus, the urine assay was performed within 6 months of collection. Frozen urine samples were thawed at room temperature, dispersed by ultrasonic waves, and filtered through a Sep-Pak C^{18} column (Mallinckrodt Baker, N.J., USA), and levels of As^{III}, As^V, MMA^V, and DMA^V were determined. A urine aliquot of 200 μl was used for the determination of arsenic species by high-performance liquid chromatography (HPLC; Waters 501, Waters Associates, Mass., USA) with columns obtained from Phenomenex (Nucleosil, Torrance, Calif., USA). The contents of inorganic arsenic and its metabolites were quantified by hydride generator-atomic absorption spectrometry (AAS) [25]. Recovery rates for As^{III}, DMA^V, MMA^V, and As^V ranged between 93.8 and 102.2% with detection limits of 0.02, 0.06, 0.07, and 0.10 μg/l, respectively. The urinary concentration

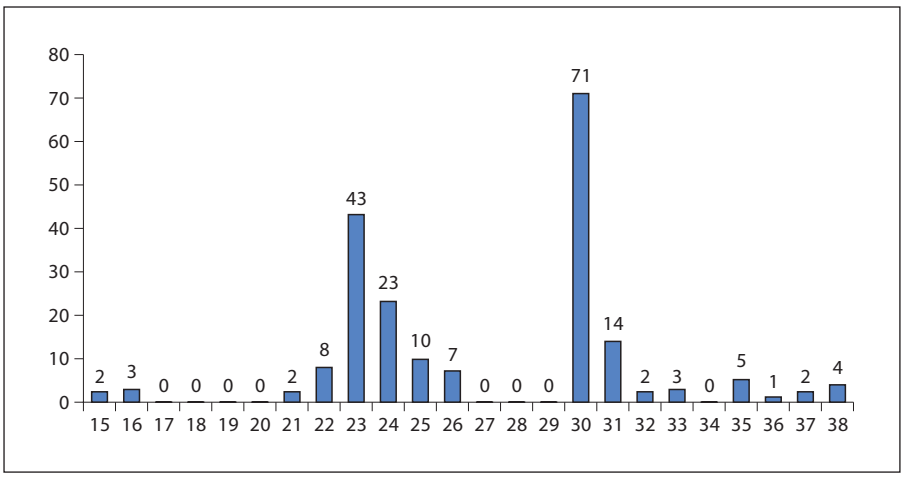

Fig. 1. Frequency distribution of HO1 $(GT)_n$ repeats (alleles = 200).

of total arsenic was normalized against urinary creatinine levels (μg/g creatinine). The standard reference material, SRM 2670, contains 480 ± 100 μg/l inorganic arsenic and was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, Md., USA). SRM 2670 was used as a quality standard and analyzed along with the urine samples. The mean ± SD value of SRM 2670 determined by our system was 507 ± 17 μg/l (n = 4). Determining the percentages of various urinary arsenic species of the total arsenic amount assessed the arsenic methylation capability. The primary methylation index (PMI) was defined as the ratio between MMA and inorganic arsenic (As^{III} + As^{V}) levels, while the secondary methylation index (SMI) was defined as the ratio between DMA and MMA [26].

Determination of HO-1 *Genetic Polymorphism*

DNA was extracted from the buffy coat using a previously described protocol [27] and sent blinded to the laboratory for genotyping. The poly $(GT)_n$ repeat of the HO-1 gene was amplified by PCR with a forward primer (5′-CCA GGC TTT TGC TCT GAG CA) and a reverse primer (5′-ACC GCA TAC CAG GGT GCC). Thermocycler conditions for amplification consisted of one cycle of 95°C for 5 min, 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s, and one cycle of 72°C for 10 min followed by holding at 4°C. The number of repeats was observed by direct sequencing (in an ABI 3100 automated DNA sequencer). All sequencing reactions used ABI standard reagents and protocols for the ABI 3100 automated DNA sequencer.

Determination of NQO1 *Genetic Polymorphism*

The proline-to-serine change at amino acid 187 of *NQO1* due to the C609T polymorphism was analyzed using a method based on polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses as described by Chio et al. [28]. In brief, *NQO1* primers (5′-TCC TCA GAG TGGCAT TCT GC-3′ and 5′-TCT CCT CAT CCT GTACCT CT-3′) were used. After initial denaturation at 95°C for 3 min, 35 cycles of 50 s at 95°C, 50 s at 55°C, and 20 s at 72°C were performed, followed by a final extension step of 5 min at 72°C. After the PCR, an aliquot of the product 10 μl was digested with 0.2 μl *Hin*fI for 3 h at 37°C, produced 195-bp bands for C/C; 195-, 151- and 44-bp bands for C/T, and 151- and 44-bp bands for T/T. These digested products were electrophoresed on a 2.5% agarose gel and visualized by 0.5 μg/ml ethidium bromide staining.

Statistical Analysis

Continuous variables are expressed as the mean ± SE. ANOVA and Scheffe's test for multiple comparison correction were applied to compare urinary arsenic profiles between various tumor grades and stages. The χ^2 test was used to determine the association of tumor grades and stages with the genotype and demographic variables. SAS version 8.2 was used for all statistical analyses.

Results

The distribution of stage and grade in UC patients did not differ by gender, blood type, marital status, educational level, or paternal and maternal ethnicity (table 1). The frequency distribution of the HO-1 $(GT)_n$ repeats in UC cases is shown in figure 1. The range of the number of HO-1 $(GT)_n$ repeats was 15–38, and the peak numbers of HO-1 $(GT)_n$ repeats were 23 and 30 repeats among UC cases. Table 2 compares the urinary arsenic profile between genders, and the NQO1 and HO-1 genotypes. Among UC cases, male subjects had an insignificantly higher total arsenic level, lower inorganic arsenic, higher MMA percentage, and higher DMA percentage than females. The urinary arsenic profiles of the various NQO1 and HO-1 genotypes did not significantly differ (table 2). To examine if various cancer stages affected the arsenic methylation capability, we performed an analysis, which showed that the methylation capability differed among patients with various tumor stages (table 3). T_3/T_4 stage cases had significantly higher inorganic arsenic and MMA levels than T_2 stage cases; they also had significantly high-

Table 1. Distribution of gender, blood type, marital status, educational level, and paternal and maternal ethnicity in urothelial cancer patients by stage and grade

	Stage				χ^2 (p value)	Grade				χ^2 (p value)
	T_a/T_1	T_2	T_3/T_4	not available		I	II	III/IV	not available	
Total number	52	14	16	18		9	46	27	18	
Gender					3.88 (0.14)					0.23 (0.88)
Male	32	5	11	12		5	28	15	12	
Female	20	9	5	6		4	18	12	6	
Blood type					3.51 (0.89)					5.55 (0.69)
A	8	4	4	5		1	8	7	5	
B	10	3	3	2		3	10	2	3	
O	23	4	8	9		4	19	13	8	
AB	1	0	0	1		0	1	0	1	
Not available	10	3	1	1		1	8	5	1	
Marital status					5.07 (0.27)					1.79 (0.77)
Single	0	0	1	0		0	1	0	0	
Married	38	11	13	13		7	33	21	14	
Divorced	0	0	0	0		0	0	0	0	
Widowed	14	3	2	5		2	12	4	4	
Not available	0	0	0	0		0	0	2	0	
Educational level					5.07 (0.74)					5.14 (0.74)
Illiterate	21	7	4	3		3	18	11	3	
Elementary school	19	4	5	12		4	15	9	12	
Junior high school	6	1	4	2		1	5	5	2	
High school	5	1	2	1		0	6	2	1	
University	1	1	1	0		1	2	0	0	
Paternal ethnicity					4.73 (0.31)					2.28 (0.58)
Fukien Taiwanese	51	14	15	18		9	45	26	18	
Hakka Taiwanese	1	0	0	0		0	1	0	0	
Mainland Chinese	0	0	1	0		0	0	1	0	
Maternal ethnicity					4.17 (0.12)					2.06 (0.37)
Fukien Taiwanese	52	14	15	18		9	46	26	18	
Hakka Taiwanese	0	0	0	0		0	0	0	0	
Mainland Chinese	0	0	1	0		0	0	1	0	

χ^2 tests excluded data not available.

er MMA and total arsenic levels than T_a/T_1 stage cases. In contrast, the methylation capability did not differ among patients with various tumor grades (table 4). The frequency distribution of HO-1 significantly differed among patients with various tumor stages (table 5).

Discussion

In this study, urinary arsenic species were used to characterize the arsenic methylation capability in UC from patients who had drunk tap water with arsenic levels of <50 μg/l. The stages of UC patients were identified as 52 at T_a/T_1, 14 at T_2, 16 at the T_3/T_4 stage, and 18 not available for staging, while 9 were at grade I, 46 at grade II, 27 at grade III/IV, and 18 were not available for grading. We found that different stages and grades had similar distributions of gender, blood type, marital status, educational level, and paternal and maternal ethnicity. In addition, we also found that inorganic arsenic; MMA, DMA, and total arsenic levels were significantly higher in T_3/T_4 patients than in T_2 or T_a/T_1 patients. This finding raises the possibility that the arsenic methylation capability increases the chance for a tumor prognosis. On the other hand, the frequency distribution of HO-1 differed in the various UC stages.

Table 2. Distribution of the urinary arsenic methylation profile in urothelial cancer patients by gender, NQO1, and HO-1 genotype

	n	Total arsenic	Percent inorganic arsenic	Percent MMA	Percent DMA	Primary methylation index	Secondary methylation index
Total number	100	26.14 ± 3.91	6.19 ± 0.99	7.52 ± 0.60	86.29 ± 1.19	2.59 ± 0.56	25.54 ± 4.60
Gender							
Male	60	29.92 ± 6.31	5.98 ± 0.76	7.62 ± 0.81	86.40 ± 1.24	2.11 ± 0.34	30.21 ± 7.24
Female	40	20.46 ± 2.29	6.52 ± 2.20	7.36 ± 0.91	86.12 ± 2.33	3.46 ± 1.44	18.21 ± 3.07
p value[a]		0.14	0.83	0.83	0.91	0.37	0.13
NQO1							
WW	22	22.00 ± 2.98	4.70 ± 0.89	7.45 ± 1.06	87.84 ± 1.58	2.29 ± 0.86	22.35 ± 7.93
WM	24	31.59 ± 7.27	7.00 ± 1.70	7.52 ± 0.86	85.48 ± 1.82	2.06 ± 0.32	25.11 ± 5.56
MM	26	18.75 ± 2.43	5.85 ± 1.52	7.57 ± 1.31	86.58 ± 2.46	3.98 ± 1.99	29.31 ± 12.48
p value[b]		0.33	0.64	0.99	0.73	0.34	0.86
HO-1							
SS	18	23.84 ± 3.78	6.35 ± 1.16	9.18 ± 1.61	84.47 ± 2.08	2.35 ± 0.54	14.42 ± 3.17
SL	62	27.99 ± 6.05	5.11 ± 0.69	7.38 ± 0.72	87.51 ± 1.21	2.11 ± 0.34	28.70 ± 6.31
LL	30	22.46 ± 4.58	9.42 ± 4.34	6.46 ± 1.43	84.12 ± 4.24	4.48 ± 2.75	24.96 ± 11.67
p value[b]		0.83	0.23	0.36	0.42	0.27	0.86

Primary methylation index = inorganic arsenic concentration/MMA concentration; secondary methylation index = MMA concentration/DMA concentration.

SS = HO-1 alleles with fewer than 28 (GT) repeats; SL = HO-1 one allele with fewer than 28 (GT) repeats, one allele with 28 or more (GT) repeats; LL = HO-1 alleles with more than 28 (GT) repeats.

[a] Student's t test; [b] ANOVA test.

Table 3. Distribution of the urinary arsenic methylation profile in urothelial cancer patients by stage

	T_a/T_1 (n = 52)	T_2 (n = 14)	T_3/T_4 (n = 16)	p value for ANOVA
Urinary arsenic species, μg/l				
Inorganic arsenic	0.96 ± 0.10	0.53 ± 0.15[a]	1.67 ± 0.46	0.01
MMA	1.65 ± 0.19[a]	0.84 ± 0.29[a]	2.96 ± 0.68	<0.01
DMA	19.15 ± 1.86	16.37 ± 3.15	46.54 ± 22.12	0.05
Total arsenic	21.75 ± 2.03[a]	17.74 ± 3.35	51.17 ± 22.43	0.03
Urinary arsenic species percentage				
Inorganic arsenic	5.76 ± 0.72	10.12 ± 6.16	7.06 ± 1.88	0.40
MMA	8.19 ± 0.80	4.66 ± 1.34	9.65 ± 1.97	0.07
DMA	86.05 ± 1.17	85.21 ± 5.89	83.29 ± 3.51	0.75
Arsenic methylation index				
Primary	2.34 ± 0.43	5.30 ± 4.36	2.38 ± 0.64	0.31
Secondary	24.59 ± 7.02	34.82 ± 16.12	16.07 ± 4.43	0.58

Primary methylation index = inorganic arsenic concentration/MMA concentration; secondary methylation index = MMA concentration/DMA concentration.

[a] ANOVA and Scheffe's test, T_a/T_1 vs.T_3/T_4 and T_2 vs.T_3/T_4.

Table 4. Distribution of the urinary arsenic methylation profile in urothelial cancer patients by grade

	Grade I (n = 9)	Grade II (n = 46)	Grade III and IV (n = 27)	p value for ANOVA
Urinary arsenic species, μg/l				
Inorganic arsenic	0.89 ± 0.30	0.90 ± 0.11	1.23 ± 0.29	0.44
MMA	1.79 ± 0.40	1.45 ± 0.20	2.32 ± 0.47	0.13
DMA	22.26 ± 4.43	19.77 ± 2.17	31.58 ± 13.36	0.50
Total arsenic	24.94 ± 4.93	22.12 ± 2.33	35.13 ± 13.61	0.45
Urinary arsenic species, %				
Inorganic arsenic	3.46 ± 1.02	6.25 ± 0.99	8.58 ± 3.17	0.42
MMA	6.98 ± 1.16	7.80 ± 0.95	8.58 ± 1.26	0.77
DMA	89.56 ± 1.63	85.95 ± 1.66	82.84 ± 3.09	0.34
Arsenic methylation index				
Primary	3.75 ± 2.04	2.20 ± 0.40	3.43 ± 1.73	0.62
Secondary	24.67 ± 9.68	29.54 ± 8.56	13.85 ± 2.98	0.39

Primary methylation index = inorganic arsenic concentration/MMA concentration; secondary methylation index = MMA concentration/DMA concentration.

Table 5. Frequency distribution of the NQO1 and HO-1 genotypes in urothelial cancer patients by stage and grade

Genotype	T_a/T_1 (n = 52)	T_2 (n = 14)	T_3/T_4 (n = 16)	χ^2 (p value)
NQO1				1.80 (0.77)
WW	12	4	5	
WM	26	6	9	
MM	14	4	2	
HO-1				11.38 (0.02)
SS	11	0	3	
SL	32	7	12	
LL	9	7	1	

Genotype	Grade I (n = 9)	Grade II (n = 46)	Grades III and IV (n = 27)	χ^2 (p value)
NQO1				3.39 (0.49)
WW	3	9	10	
WM	5	23	12	
MM	1	14	5	
HO-1				1.23 (0.87)
SS	4	1	9	
SL	10	7	28	
LL	4	1	9	

SS = HO-1 alleles with fewer than 28 (GT) repeats; SL = HO-1 one allele with fewer than 28 (GT) repeats, one allele with 28 or more (GT) repeats; LL = HO-1 alleles with more than 28 (GT) repeats.

Since tumor grade and stage are considered to be the main prognostic factors in bladder cancer, we investigated whether these prognosticators have an association with HO-1 activity in UC. The induction of HO-1 represents a cytoprotective defense mechanism against oxidative insults, and a high expression of HO-1 is found with malignant tumors [29]. The role of HO-1 is advantageous for tumor progression and survival [30, 31]. $(GT)_n$ repeats were identified in the proximal region of the *HO-1* promoter. Reports have indicated that these microsatellites are highly polymorphic, and longer $(GT)_n$ repeats exhibit lower HO-1 transcriptional activity [32]. A recent Taiwanese study showed that subjects carrying longer $(GT)_n$ repeats in the *HO-1* promoter are associated with risks of areca (betel nut, *Areca catechu*)-related oral squamous cell carcinoma [33]. Preliminary evidence from the present study indicates that the different distributions of *HO-1* promoter polymorphism in the various stages of UC might reflect some impact on tumor progression. Other studies have described risk factors being mediated by stage and grade of bladder cancer as follows. Aberrant expression of heat shock protein-70 by bladder cancer patients occurs frequently and indicates a more-aggressive phenotype, which has been correlated with advanced grades and stages and poor outcomes for patients with bladder cancer [34]. These results suggest that a genetic predisposition might contribute to this phenomenon. A recent study reported that the proportion of tumor samples with *p53* mutations and P53 immunopositivity

strongly increased with both stage and grade of bladder cancer, but not with arsenic exposure or smoking [35]. Methylation silencing of two tumor suppressor genes, *RASSFIA* and *PRSS3,* was significantly associated with an invasive tumor stage, and showed increasing trends in the relative risk estimates of promoter methylation from non-invasive, low-grade tumors through the invasive stage, suggesting that these alterations may occur late in carcinogenesis of the bladder [36]. Additional genotypic surveys using more samples together with HO-1 expression profiles are required to gain further insights into the functional importance of HO-1 in UC cancer progression.

Bladder tumors in patients with higher levels of arsenic exposure showed higher levels of chromosomal instability, and most of the chromosomal alterations associated with arsenic exposure were also associated with tumor stage and grade [37]. This suggests that tumors in UC patients with higher urinary arsenic species might behave more aggressively than those in patients with lower levels of urinary arsenic species. Until recently, the arsenic methylation process ($As^{V} \rightarrow As^{III} \rightarrow MMA^{V} \rightarrow MMA^{III} \rightarrow DMA^{V} \rightarrow DMA^{III}$) [38] was thought to be a detoxification pathway because the major methylated metabolites MMA^{V} and DMA^{V} are more readily excreted and less toxic than inorganic arsenic [39]. Nevertheless, the minor trivalent methylated arsenicals, MMA^{III} and DMA^{III}, are more potent than either As^{III} or As^{V} in cytotoxicity [40], genotoxicity [41, 42], and inhibition of enzymes with antioxidant functions [43, 44]. An individual's methylation capacity plays an important role in determining the susceptibility to the adverse health effects of arsenic, especially MMA% or the MMA/DMA ratio and arsenic-related skin cancer and bladder cancer [11, 12]. A recent report indicated that individual methylation patterns remain fairly stable over time [45], suggesting that the predominant factors controlling this process are either genetically influenced or related to environmental factors such as long-term dietary patterns or smoking habits that also remain stable over time. Several studies have shown that inherited genetic traits might play important roles in determining individual arsenic methylation capabilities [46, 47]. Previous studies showed that changes in arsenic methylation observed in patients with liver insufficiency may have resulted from a depletion of liver glutathione and/or a reduction in glutathione transferase rather than from reduced uptake of inorganic arsenic by the liver [48, 49]. This finding may be proven by the *GSTM1* null genotype showing a correlation between this genotype and the stage of bladder cancer [50]. In contrast, in this study, the MMA percentage in the T_3/T_4 stage of UC was borderline significantly higher than those of the T_2 and T_a/T_1 stages, suggesting that increased urinary excretion of unknown substances metabolized by GSTM1 may promote cancer progression in patients with bladder cancer [51]. In conclusion, evaluating higher-stage UC patients with higher urinary arsenic concentrations regardless of whether they had been exposed to arsenic from the drinking water requires further large-scale investigations.

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References

1 Department of Health ROC: Cancer Registry Annual Report Republic of China, 1999; in Department of Health ROC (ed): Taipei, Department of Health, Executive, Yuan 1998, 2002.
2 Chou YH, Huang CH: Unusual clinical presentation of upper urothelial carcinoma in Taiwan. Cancer 1999;85:1342–1344.
3 Chen CJ, Kuo TL, Wu MM: Arsenic and cancers. Lancet 1988;i:414–415.
4 Chiou HY, Chiou ST, Hsu YH, Chou YL, Tseng CH, Wei ML, Chen CJ: 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; 153:411–418.
5 Lee SH, Lin JS, Tzai TS, Chow NH, Tong YC, Yang WH, Chang CC, Cheng HL: Prognostic factors of primary transitional cell carcinoma of the upper urinary tract. Eur Urol 1996; 29:266–270.
6 Yang MH, Chen KK, Yen CC, Wang WS, Chang YH, Huang WJ, Fan FS, Chiou TJ, Liu JH, Chen PM: Unusually high incidence of upper urinary tract urothelial carcinoma in Taiwan. Urology 2002;59:681–687.
7 Lee SE, Park MS: Prognostic factors for survival in patients with transitional cell carcinoma of the bladder: evaluation by histopathologic grade, pathologic stage and flow-cytometric analysis. Eur Urol 1996;29: 193–198.
8 Zieger K, Wolf H, Olsen PR, Hojgaard K: Long-term survival of patients with bladder tumours: the significance of risk factors. Br J Urol 1998;82:667–672.
9 Andreae MO: Determination of arsenic species in natural waters. Anal Chem 1977;49: 820–823.
10 Vahter M, Marafante E, Dencker L: Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. Arch Environ Contam Toxicol 1984;13:259–264.
11 Hsueh YM, Chiou HY, Huang YL, Wu WL, Huang CC, Yang MH, Lue LC, Chen GS, Chen CJ: Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. Cancer Epidemiol Biomarkers Prev 1997;6:589–596.

12 Chen YC, Su HJ, Guo YL, Hsueh YM, Smith TJ, Ryan LM, Lee MS, Christiani DC: Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 2003;14:303–310.
13 Lind C, Cadenas E, Hochstein P, Ernster L: DT-diaphorase: purification, properties, and function. Methods Enzymol 1990;186:287–301.
14 Cadenas E: Antioxidant and prooxidant functions of DT-diaphorase in quinone metabolism. Biochem Pharmacol 1995;49:127–140.
15 Jaiswal AK, McBride OW, Adesnik M, Nebert DW: Human dioxin-inducible cytosolic NAD(P)H:menadione oxidoreductase: cDNA sequence and localization of gene to chromosome 16. J Biol Chem 1988;263:13572–13578.
16 Lafuente MJ, Casterad X, Trias M, Ascaso C, Molina R, Ballesta A, Zheng S, Wiencke JK, Lafuente A: NAD(P)H:quinone oxidoreductase-dependent risk for colorectal cancer and its association with the presence of K-ras mutations in tumors. Carcinogenesis 2000;21:1813–1819.
17 Lin P, Hsueh YM, Ko JL, Liang YF, Tsai KJ, Chen CY: Analysis of NQO1, GSTP1, and MnSOD genetic polymorphisms on lung cancer risk in Taiwan. Lung Cancer 2003;40:123–129.
18 Park SJ, Zhao H, Spitz MR, Grossman HB, Wu X: An association between NQO1 genetic polymorphism and risk of bladder cancer. Mutat Res 2003;536:131–137.
19 Maines MD: Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 1988;2:2557–2568.
20 Abraham NG, Drummond GS, Lutton JD, Kappas A: The biological significance and physiological role of heme oxygenase. Cell Physiol Biochem 1996;6:129–168.
21 Maines MD: Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 1988;2:2557–2568.
22 Kutty RK, Kutty G, Rodriguez IR, Chader GJ, Wiggert B: Chromosomal localization of the human heme oxygenase genes: heme oxygenase-1 (HMOX1) maps to chromosome 22q12 and heme oxygenase-2 (HMOX2) maps to chromosome 16p13.3. Genomics 1994;20:513–516.
23 Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H: Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 2000;66:187–195.
24 Chen YC, Amarasiriwardena CJ, Hsueh YM, Christiani DC: Stability of arsenic species and insoluble arsenic in human urine. Cancer Epidemiol Biomarkers Prev 2002;11:1427–1433.
25 Hsueh YM, Huang YL, Huang CC, Wu WL, Chen HM, Yang MH, Lue LC, Chen CJ: Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. J Toxicol Environ Health [A] 1998;54:431–444.
26 Tseng CH, Huang YK, Huang YL, Chung CJ, Yang MH, Chen CJ, Hsueh YM: Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. Toxicol Appl Pharmacol 2005;206:299–308.
27 Lee HH, Chao HT, Ng HT, Choo KB: Direct molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia. J Med Genet 1996;33:371–375.
28 Choi JY, Lee KM, Cho SH, Kim SW, Choi HY, Lee SY, Im HJ, Yoon KJ, Choi H, Choi I, Hirvonen A, Hayes RB, Kang D: CYP2E1 and NQO1 genotypes, smoking and bladder cancer. Pharmacogenetics 2003;13:349–355.
29 Goodman AI, Choudhury M, da Silva JL, Schwartzman ML, Abraham NG: Overexpression of the heme oxygenase gene in renal cell carcinoma. Proc Soc Exp Biol Med 1997;214:54–61.
30 Fang J, Sawa T, Akaike T, Akuta T, Sahoo SK, Khaled G, Hamada A, Maeda H: In vivo antitumor activity of pegylated zinc protoporphyrin: targeted inhibition of heme oxygenase in solid tumor. Cancer Res 2003;63:3567–3574.
31 Malaguarnera L, Quan S, Pilastro MR, Abraham NG, Kappas A: Diminished heme oxygenase potentiates cell death: pyrrolidinedithiocarbamate mediates oxidative stress. Exp Biol Med (Maywood) 2003;228:459–465.
32 Kaneda H, Ohno M, Taguchi J, Togo M, Hashimoto H, Ogasawara K, Aizawa T, Ishizaka N, Nagai R: Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors. Arterioscler Thromb Vasc Biol 2002;22:1680–1685.
33 Chang KW, Lee TC, Yeh WI, Chung MY, Liu CJ, Chi LY, Lin SC: Polymorphism in heme oxygenase-1 (HO-1) promoter is related to the risk of oral squamous cell carcinoma occurring on male areca chewers. Br J Cancer 2004;91:1551–1555.
34 Syrigos KN, Harrington KJ, Karayiannakis AJ, Sekara E, Chatziyianni E, Syrigou EI, Waxman J: Clinical significance of heat shock protein-70 expression in bladder cancer. Urology 2003;61:677–680.
35 Moore LE, Smith AH, Eng C, DeVries S, Kalman D, Bhargava V, Chew K, Ferreccio C, Rey OA, Hopenhayn C, Biggs ML, Bates MN, Waldman FM: P53 alterations in bladder tumors from arsenic and tobacco exposed patients. Carcinogenesis 2003;24:1785–1791.
36 Marsit CJ, Karagas MR, Danaee H, Liu M, Andrew A, Schned A, Nelson HH, Kelsey KT: Carcinogen exposure and gene promoter hypermethylation in bladder cancer. Carcinogenesis 2006;27:112–116.
37 Moore LE, Smith AH, Eng C, Kalman D, DeVries S, Bhargava V, Chew K, Moore D, Ferreccio C, Rey OA, Waldman FM: Arsenic-related chromosomal alterations in bladder cancer. J Natl Cancer Inst 2002;94:1688–1696.
38 Kitchin KT: Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 2001;172:249–261.
39 Gebel TW: Arsenic methylation is a process of detoxification through accelerated excretion. Int J Hyg Environ Health 2002;205:505–508.
40 Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ: Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch Toxicol 2000;74:289–299.
41 Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ, Kligerman AD: Methylated trivalent arsenic species are genotoxic. Chem Res Toxicol 2001;14:355–361.
42 Nesnow S, Roop BC, Lambert G, Kadiiska M, Mason RP, Cullen WR, Mass MJ: DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. Chem Res Toxicol 2002;15:1627–1634.
43 Lin S, Del Razo LM, Styblo M, Wang C, Cullen WR, Thomas DJ: Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. Chem Res Toxicol 2001;14:305–311.
44 Styblo M, Serves SV, Cullen WR, Thomas DJ: Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem Res Toxicol 1997;10:27–33.
45 Steinmaus C, Yuan Y, Kalman D, Atallah R, Smith AH: Intraindividual variability in arsenic methylation in a US population. Cancer Epidemiol Biomarkers Prev 2005;14:919–924.
46 Chung JS, Kalman DA, Moore LE, Kosnett MJ, Arroyo AP, Beeris M, Mazumder DN, Hernandez AL, Smith AH: Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. Environ Health Perspect 2002;110:729–733.
47 Vahter M: Mechanisms of arsenic biotransformation. Toxicology 2002;181–182:211–217.
48 Buchet JP, Geubel A, Pauwels S, Mahieu P, Lauwerys R: The influence of liver disease on the methylation of arsenite in humans. Arch Toxicol 1984;55:151–154.
49 Buchet JP, Lauwerys R: Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicol Appl Pharmacol 1987;91:65–74.
50 Georgiou I, Filiadis IF, Alamanos Y, Bouba I, Giannakopoulos X, Lolis D: Glutathione S-transferase null genotypes in transitional cell bladder cancer: a case-control study. Eur Urol 2000;37:660–664.
51 Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun WJ, Kim WJ: Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. Urology 2005;65:70–75.