Research Article

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Dose–Response Relationship between Inorganic Arsenic Exposure and Lung Cancer among Arseniasis Residents with Low Methylation Capacity

Kuang-Hung Hsu[1,2], Ke-Hung Tsui[2], Ling-I Hsu[3], Hung-Yi Chiou[4], and Chien-Jen Chen[3]

Abstract

Background: Exposure to inorganic arsenic (InAs) has been documented as a risk factor for lung cancer. This study examined the association between InAs exposure, its metabolism, and lung cancer occurrence.

Methods: We followed 1,300 residents from an arseniasis area in Taiwan, determined urinary InAs metabolites, and identified 39 lung cancer cases. Cox proportional hazards model was performed.

Results: The results demonstrated that participants with either the primary methylation index [monomethylarsonic acid (MMA)/InAs] or the secondary methylation index [dimethylarsenic acid (DMA)/MMA] lower than their respective median values were at a higher risk of lung cancer (HRs from 3.41 to 4.66) than those with high methylation capacity. The incidence density of lung cancer increased from 79.9/100,000 (year^{-1}) to 467.4/100,000 (year^{-1}) for residents with low methylation capacity and from 0 to 158.5/100,000 (year^{-1}) for residents with high methylation capacity when the arsenic exposure dose increased from 2 to 10 ppb to $\geq$200 ppb, respectively. The analyses revealed a dose–response relationship between lung cancer occurrence and increasing arsenic concentrations in drinking water as well as cumulative arsenic exposure (monotonic trend test; $P < 0.05$ and $P < 0.05$, respectively) among the residents with low methylation capacity. The relationship between arsenic exposure and lung cancer among high methylators was not statistically significant.

Conclusions: Hypomethylation responses to InAs exposure may dose dependently increase lung cancer occurrence.

Impact: The high-risk characteristics observed among those exposed should be considered in future preventive medicine and research on arsenic carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 26(5); 756–61. ©2016 AACR.*

Introduction

Exposure to inorganic arsenic (InAs) is considered an etiologic factor for mortality of cancers and vascular diseases in humans (1). Cancers of skin and other internal organs, including lung, liver, bladder, and kidney, are associated with InAs exposure (2, 3). Recent studies from Bangladesh have demonstrated an association between InAs exposure and the onset of lung and other internal organ cancers (4, 5).

People metabolize exposed InAs through two steps of methylation. In general, arsenate (As^{+5}) was reduced into arsenite (As^{+3}) and then methylated into monomethylarsonic acid (MMA^{+5}) in the first step of methylation. Subsequently, monomethylarsonic acid (MMA^{+5}) was reduced into monomethylarsonous acid (MMA^{+3}) and in turn methylated into dimethylarsenic acid (DMA^{+5}) in the second methylation step. They are detected in urine (6, 7). Residents in arseniasis areas with low methylation capacity are likely to develop skin and bladder cancers (8–11). A study conducted in Taiwan's arseniasis area demonstrated that the observed adverse health effects were associated with chronic arsenic exposure and its metabolism pattern (11). Previous studies have indicated that MMA, particularly MMA^{+3}, may be the primary toxic species and defined it as the biologically effective doses of InAs exposure in humans (12, 13). However, evidence obtained from case–control studies reporting an association between InAs metabolism and arsenic exposure at low concentrations and lung cancer is limited (14, 15). The metabolic patterns from case–control studies are assessed after cancer diagnosis and temporality cannot be assured, as cancer could potentially in theory change InAs metabolism. Therefore, an in-depth cohort design–based examination of the effects of arsenic methylation capacity and arsenic exposure doses on inducing lung cancer is warranted.

Mixed results were obtained for InAs metabolism patterns, primary methylation index (PMI) and secondary methylation index (SMI), calculated by using ratios of MMA to InAs and DMA to MMA, respectively, and different manifestations of arsenic

[1]Laboratory for Epidemiology, Department of Health Care Management and Healthy Aging Research Center, Chang Gung University, Taoyuan City, Taiwan. [2]Department of Urology, Chang Gung Memorial Hospital, Chang Gung University, Taipei, Taiwan. [3]Genomics Research Center, Academia Sinica, Taipei, Taiwan. [4]School of Public Health, College of Public Health and Nutrition, Taipei Medical University, Taipei City, Taiwan.

Corresponding Authors: Hung-Yi Chiou, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei City 110, Taiwan. Phone: 8862-2377-9189; Fax: 8862-2377-9188; E-mail: hychiou@tmu.edu.tw; and Chien-Jen Chen, Genomics Research Center, Academia Sinica, No. 128 Academia Road, Section 2, Nankang, Taipei City 115, Taiwan. Phone: 886-3-2789-8781; Fax: 886-2-2785-3208; E-mail: chencj@gate.sinica.edu.tw

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toxicities (7, 12, 16–18). One study demonstrated an association between InAs metabolism-related genes and lung cancer occurrence (19), which is valuable for predicting high-risk factors and regulating arsenic carcinogenesis. However, the dose–response relationship between InAs exposure and its metabolism pattern and the risk of lung cancer has not been adequately addressed in humans (20). Therefore, this long-term follow-up study examined the role of InAs metabolic patterns in lung cancer occurrence among the residents of an arseniasis area by assessing the dose–response relationship.

Materials and Methods

Study participants

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (Taipei, Taiwan; 100-2839C). On the basis of our previously set up cohort in arseniasis areas, 1,300 residents who were exposed to arsenic-contaminated (>2 ppb) well water and were eligible for a urinary InAs speciation analysis were defined as the study cohort. In the 1992 to 1995, residents of an arseniasis area in northeast Taiwan were recruited for this study because they were exposed to arsenic-contaminated water since birth (21). This prospective follow-up study was conducted between August 1995 and December 2011, and the average follow-up period was 15.0 years. In the years following data collection, the local government implemented a tap water supply program, which provided nearly 100% of all tap water in the study area by the late 1990s. According to the initial questionnaire outcomes and laboratory test results, the residents were exposed to arsenic-contaminated drinking water for an average of 40 years, with a mean arsenic concentration of 99.5 ppb (0–3,592 ppb).

Data collection

Basic demographic characteristics, namely age, sex, marital status, education, occupation, cigarette smoking, alcohol drinking, coffee drinking, tea drinking, other chemical exposure and medication drug usage, and duration of well-water exposure, were assessed using a questionnaire. Lung cancer diagnosis data were obtained from the National Cancer Registration Database and verified against the insurance claims data. International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code 162.xx was used to define lung cancer. As of the end of 2011, 39 incident cases of lung cancer were identified.

Arsenic speciation and metabolism pattern classification

Underground water and urine samples were collected only at the residence at the time of recruitment and were stored at −20°C until assays. Arsenic concentration in the water was determined immediately after sample collection. Urinary arsenic speciation was performed through HPLC for separating the arsenic species, followed by inductively coupled plasma mass spectrophotometry for determining the concentrations of the separated arsenic species. The detection limits of urinary InAs metabolites were 0.4, 1.4, 1.4, and 1.0 ppb for As^{+3}, As^{+5}, MMA, and DMA, respectively. A spiking analysis yielded an average recovery rate of 95.18% to 100.03%. SRM 2670a, a standard reference material, was used for validation, and the values were calculated within the suggested range. According to our review of relevant literature, the InAs metabolism pattern was further indicated by PMI, calculated as MMA/(As^{+3} + As^{+5}), and SMI, calculated as DMA/MMA (7–9, 18).

Statistical analyses

Numerical variables with apparent skewness were displayed as the median value (first and third quartiles), and the nonparametric Wilcoxon rank sum test was used for comparing the differences between the study groups. The Cox proportional hazards model was used for determining the strength of the association between the study variables and lung cancer occurrence during examining and for adjustment for the effects from other selected variables. Considering the collinearity among arsenic concentration, duration of arsenic exposure, and cumulative arsenic exposure, we constructed various regression models for assessments. The results were expressed as multivariate adjusted HRs and the corresponding 95% confidence intervals (CI). The InAs metabolism patterns were further categorized on the basis of the medians of PMI and SMI into four groups: low PMI/low SMI, low PMI/high SMI, high PMI/low SMI, and high PMI/high SMI. For estimating the arsenic exposure doses, water arsenic concentrations were categorized using two methods: categorization according to literature review (2–10, 10.01–100, 100.01–200, and >200 ppb) and statistical categorization based on quartiles (2.01–14.64, 14.65–35.62, 35.63–102.27, and >102.27 ppb for Q1, Q2, Q3, and Q4, respectively). The cumulative arsenic exposure was categorized into four quartiles (≤0.441, 0.442–1.068, 1.069–3.185, >3.185 ppm-years for Q1, Q2, Q3, and Q4, respectively). The water arsenic concentrations at schools, away from home jobs, and at any other lifetime residences were unknown among the study subjects. Therefore, the arsenic exposure metrics, including water arsenic concentration, and cumulative arsenic exposure are subject to variation. The low methylation capacity group comprised those whose PMI or SMI was lower than their respective median values, and the high methylation capacity group comprised those whose PMI and SMI were higher than their respective median values after examining the dose–response relationship. Data were analyzed using SAS 9.30 (SAS Institute) and SPSS 18.0 (SPSS, Inc.).

Results

The median age was 56.12 years (40.03–87.86 years). The risk of lung cancer was significantly higher in the older age group (HR = 5.27; 95% CI, 2.32–11.94) than in the younger age group. Lung cancer occurrence was higher among male participants (HR = 3.25; 95% CI = 1.58–6.67) than among female participants. Lung cancer occurrence was higher among unmarried participants (HR = 2.23; 95% CI, 0.949–5.34) than among married participants. Participants with junior high school education had a higher risk of lung cancer (HR = 3.30; 95% CI, 1.46–7.47) than did those with elementary and lower levels of education. The risk of lung cancer was higher among participants with occupation in government, military, teaching, and public services (HR = 2.53; 95% CI, 1.25–5.12) than among the reference group (labor workers and business persons). Participants with a smoking habit (HR = 5.77; 95% CI, 2.74–12.15) and alcohol drinking habit (HR = 2.52; 95% CI, 1.30–4.91) had higher risks of lung cancer than the ones otherwise (Table 1).

Water arsenic concentration, duration of arsenic exposure, and cumulative arsenic exposure (47.20 ppb, 45.00 years, and 2.18 ppm-years, respectively) were significantly higher in the lung cancer group than in the noncancer control group (35.04 ppb, 39.00 years, and 1.03 ppm-years, respectively; $P = 0.007$, 0.016, and <0.001, respectively). The percentages of InAs and MMA

Table 1. Association between demographic characteristics and occurrence of lung cancer among the study participants

	Participants *N* (%)	Follow-up person-year	Number of cancers	Incidence density	HR	95% CI of HR
Age						
≤56.115 years	650 (50.0)	10,465.67	7	0.0007	1	
>56.115 years	650 (50.0)	9,139.96	32	0.0035	5.27	2.32–11.94
Sex						
Female	667 (51.3)	10,354.76	10	0.0010	1	
Male	633 (48.7)	9,250.87	29	0.0031	3.25	1.58–6.67
Marital status						
Single	112 (8.6)	1,480.29	6	0.0041	2.23	0.94–5.34
Married	1,188 (91.4)	18,125.34	33	0.0018	1	
Education						
Elementary and lower	516 (39.7)	7,690.56	7	0.0009	1	
Junior high	704 (54.2)	10,646.33	32	0.0030	3.30	1.46–7.47
Senior high and higher	80 (6.2)	1,268.74	0	0	0	—
Occupation						
Unemployment and house keepers	7 (0.5)	106.67	0	0	0	—
Government, military, teacher, public services	553 (42.5)	8,107.44	26	0.0032	2.53	1.25–5.12
Agriculture, forestry, fisher, livestock	174 (13.4)	2,729.21	2	0.0007	0.58	0.13–2.6
Labor workers and business person	566 (43.5)	8,662.31	11	0.0013	1	
Cigarette smoking						
Yes	500 (38.5)	7,194.84	30	0.0042	5.77	2.74–12.15
No	800 (61.5)	12,410.79	9	0.0007	1	
Alcohol consumption						
Yes	228 (17.5)	3,247.92	13	0.0040	2.52	1.30–4.91
No	1,072 (82.5)	16,357.71	26	0.0016	1	

Abbreviation: CI, confidence interval.

(MMA%) in the urinary InAs metabolites profile were not statistically significantly higher in the lung cancer group (10.41% and 10.99%, respectively) than in the noncancer control group (9.05% and 9.56%, respectively; $P = 0.179$ and 0.296, respectively). Moreover, the percentages of DMA in the urinary InAs metabolites profile, PMI, and SMI were not statistically significantly lower (76.58%, 0.89%, and 6.65%, respectively; $P = 0.172$, 0.383, and 0.162, respectively) in the lung cancer group than in the noncancer control group (80.33%, 1.02%, and 8.13%, respectively; Table 2).

After adjustment for age, sex, education level, marital status, occupation, cigarette smoking, alcohol drinking habits, and InAs metabolism patterns, the Cox regression analyses revealed a significant association between arsenic concentration in water (each 50 ppb increment: HR = 1.03; 95% CI, 1.00–1.07) and cumulative arsenic exposure (HR = 1.02; 95% CI, 1.01–1.04), and lung cancer occurrence. A 3.41- to 4.66-fold increase in lung cancer occurrence was observed among participants with either PMI or SMI lower than their respective median values compared with those with both PMI and SMI higher than their respective median values ($P = 0.045$–0.110; Table 3).

The association between arsenic exposure and occurrence of lung cancer showed a dose–response relationship across various exposure doses in the low methylation capacity group. The incidence densities of lung cancer were 0.000799, 0.002277, 0.002646, and 0.004674 $year^{-1}$ among the participants with low methylation capacity exposed to InAs concentrations of 2–10, 10.01–100, 100.01–200, and >200 ppb, respectively ($P = 0.005$,

Table 2. InAs exposure, urinary arsenic metabolites, and occurrence of lung cancer

	Lung cancer		Noncancer controls		
Variables	**Median**	**(Q1, Q3)**	**Median**	**(Q1, Q3)**	***P*[a]**
Arsenic exposures					
Arsenic concentration in water (ppb)	47.20	(27.29, 285.17)	35.04	(14.28, 98.35)	0.007
Duration of arsenic exposure (years)	45.00	(31.00, 60.00)	39.00	(30.00, 50.00)	0.016
Cumulative arsenic exposure (ppm-years)	2.18	(0.98, 9.05)	1.03	(0.43, 3.11)	<0.001
Urinary InAs metabolites					
Total (ppb)	69.37	(32.50, 126.50)	61.56	(37.59, 105.98)	0.398
InAs (InAs = As^{+3} + As^{+5}; ppb)	7.24	(3.80, 13.17)	5.28	(3.11, 9.78)	0.08
MMA (ppb)	7.02	(3.01, 20.67)	5.67	(2.97, 10.77)	0.289
DMA (ppb)	53.00	(25.12, 95.50)	48.33	(27.36, 85.81)	0.432
Arsenic metabolism patterns					
InAs%	10.41	(6.68, 15.69)	9.05	(5.44, 15.09)	0.179
MMA%	10.99	(7.11, 15.23)	9.56	(6.28, 13.81)	0.296
DMA%	76.58	(70.16, 84.04)	80.33	(70.57, 86.14)	0.172
PMI = MMA/InAs	0.89	(0.53, 1.52)	1.02	(0.62, 1.71)	0.383
SMI = DMA/MMA	6.65	(4.61, 11.60)	8.31	(5.21, 13.25)	0.162

[a]Performed using the Wilcoxon rank sum test.

Table 3. Effects of arsenic exposure and urinary arsenic metabolism pattern on occurrence of lung cancer among the residents in the arseniasis area

		Model[a] I HR (95% CI)	Model[a] II HR (95% CI)	Model[a] III HR (95% CI)
Arsenic concentration (ppb; each 50 increment)		1.03 (1.00–1.07)	—	—
Duration of arsenic exposure (y; each increment)		—	1.02 (1.00–1.04)	—
Cumulative arsenic exposure (ppm-y; each increment)		—	—	1.02 (1.01–1.04)
Urinary arsenic metabolism pattern				
PMI (median low/high)	SMI (median low/high)			
High	High	1.00	1.00	1.00
High	Low	3.44 (0.77–15.33)	3.77 (0.85–16.82)	3.41 (0.76–15.21)
Low	High	4.25 (0.95–19.06)	4.66 (1.04–20.96)	4.32 (0.97–19.37)
Low	Low	3.98 (0.88–17.98)	4.23 (0.94–19.09)	3.72 (0.82–16.90)

Abbreviation: CI, confidence interval.

[a]Multiple Cox regression analysis adjusted for age, sex, education, occupation, marriage, cigarette smoking, and alcohol drinking.

monotonic trend test; Fig. 1A). The incidence densities of lung cancer were 0.000807, 0.002332, 0.002662, and 0.003763 year^{-1} among the participants with low methylation capacity exposed to InAs in the first, second, third, and fourth quartiles, respectively ($P = 0.010$, monotonic trend test; Fig. 1B). Furthermore, the incidence densities of lung cancer were 0.000787, 0.002118, 0.002628, and 0.004076 year^{-1} among the participants with low methylation capacity exposed to cumulative arsenic exposure in the first, second, third, and fourth quartiles, respectively ($P = 0.010$, monotonic trend test; Fig. 1C). The trend incidence density of lung cancer was not statistically significant among the participants with high methylation capacity in the three models ($P = 0.434$–0.614).

Discussion

This cohort study on the residents of arseniasis areas showed that InAs exposure, measured as arsenic concentration and cumulative arsenic exposure, has a dose–response relationship with an increased risk of lung cancer. Our findings extend the results of previous cohort studies on the association among high InAs exposure, arsenic metabolism pattern, and selective cancers

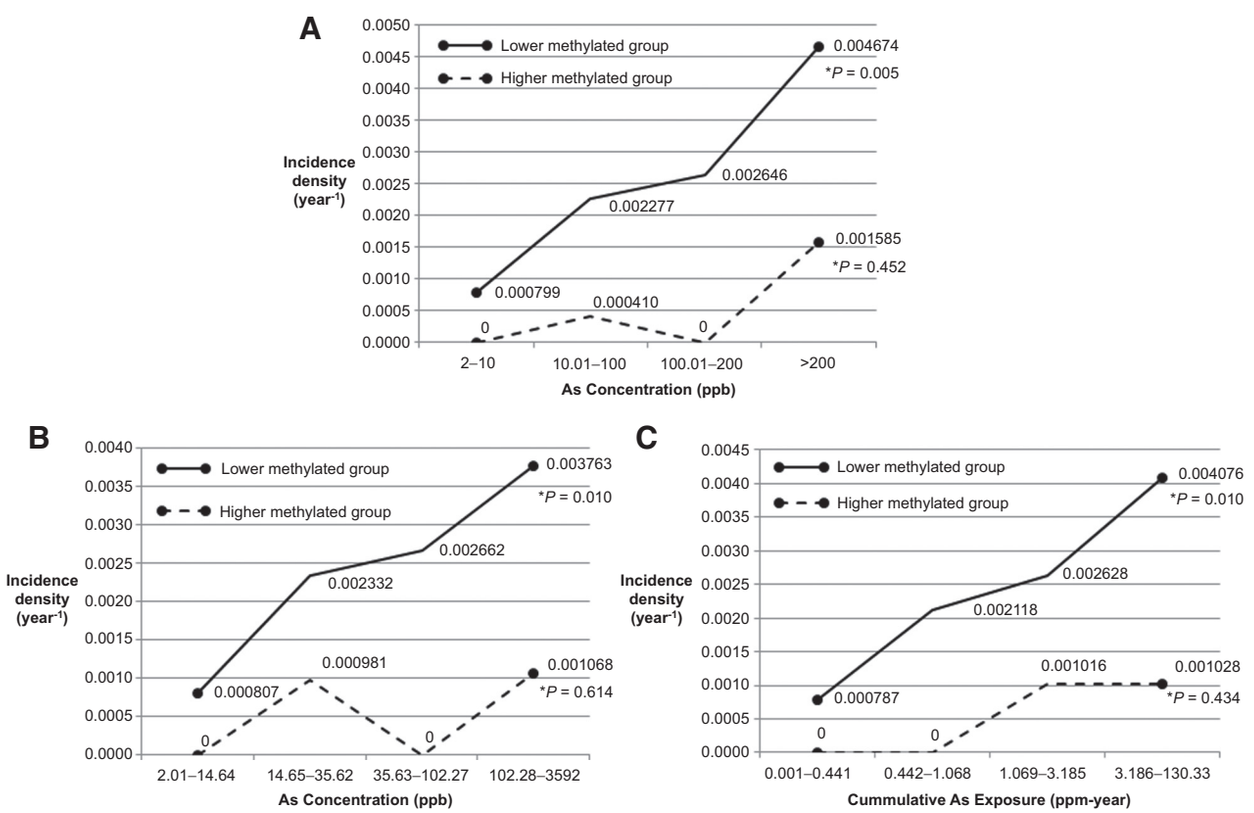

Figure 1.

Relationship between arsenic exposure and the incidence density of lung cancer. Source: authors' analysis. **A,** Arsenic concentration in water classified according to administrative dose. **B,** Arsenic concentration in water classified according to quartile. **C,** Cumulative arsenic exposure, ppm-years, according to quartile.

(8–10). These results were obtained by analyzing the associations among arsenic exposure metrics, InAs methylation patterns, and occurrence of lung cancer and provide evidence that people with low InAs methylation capacity have a prospectively high risk of the cancer. In addition, residents in arseniasis areas with low methylation capacity exposed to InAs concentrations ranging from 2 ppb to >200 ppb have a dose–response relationship with the risk of lung cancer. The finding may provide reference for future studies in discussing arsenic carcinogenesis among subjects with different susceptibility and arsenic exposure regimens (20).

Variability of arsenic carcinogenesis in people with different InAs methylation capacity is one of the main topics requiring extensive discussion. Researchers have proposed that high methylation capacity is a protective mechanism against cancer development (22). Although contradictions were found in studies using different biological systems or organs as the primary manifestations, our data show that participants with low methylation capacity are at a high risk of lung cancer. These results are consistent with previous documents indicating that InAs or MMA^{+3} accumulation was a biomarker of adverse health effects in humans (14, 22, 23). Moreover, previous studies demonstrating a positive association between increasing tertile of MMA% and arsenic carcinogenesis in the lungs were consistent with our findings using PMI and SMI as biomarkers for lung cancer (13, 14, 23).

The percentages of urinary arsenic metabolites were 9.02%–10.33%, 9.72%–11.24%, and 76.61%–80.08% for InAs, MMA, and DMA, respectively; these are within the range summarized elsewhere (14, 24), indicating that despite the different ethnicities and lifestyles of our participants, the methylation profiles were still consistent with findings reported by other studies on those with a history of long-term InAs exposure. However, the variability among the InAs methylation capacity, which exerts diverse carcinogenic effects, remains unanswered. MMA is more cytotoxic and genotoxic than As^{+3} and As^{+5} are, suggesting that the oxidation state of the methylated arsenicals is crucial in the manifestation of their cytotoxic and genotoxic effects. A recent study showed that MMA% is a potential marker of cancer mortality (25). However, we demonstrated that either a low PMI or SMI modulates arsenic carcinogenesis in the lungs. Arsenic is possibly more hazardous to hosts with methyl donor or glutathione depletion (26), as observed in people with low methylation capacity, whereas the accumulation of more toxic arsenicals results in subsequent genotoxic events, such as induction of oxidative stress, interference of signal transduction, and gene expression (27). An animal model study on arsenic carcinogenesis affirmed that the methyl group exhaustion caused by sustainable InAs methylation results in low methylation capacity and a high risk of carcinogenesis under extensive dose and InAs exposure duration (20).

The trivalent methylated arsenic species *per se* are more toxic than the premethylated inorganic compounds. A slow methylation process increases the accumulation of premethylated arsenic compounds, such as As^{+3}, MMA^{+3}, and DMA^{+3}, leading to the inhibition of cysteine-containing enzymes, cellular toxicity, genotoxicity, and clastogenicity (28). The methylated trivalent arsenicals directly or indirectly induce DNA damage (29). In animal models, MMA^{+3} or DMA^{+5} exposure leads to carcinogenesis in the lung. In addition, highly reactive intermediate MMA^{+3} has been reported to induce severe cytotoxicity, such as in hepatocytes (30). However, the direct measurement of MMA^{+3} is not practical in human urine now due to the unstable state and rapidly oxidation to MMA^{+5}. Fortunately, a single measurement of the arsenic metabolism in urine is expected to represent one's long-term methylation status (31) in which PMI and SMI are acceptable for indicating one's methylation capacity and hazards to health (7). Our results explain that low methylation capacity indicated by either low PMI or low SMI may cause arsenic carcinogenesis by accumulating premethylated arsenic compounds.

The strength of the association between arsenic exposure and cancer risk varies across populations and tissues and organs, and the reasons for this varying carcinogenic potency are yet unknown. The underlying mechanisms of arsenic carcinogenesis in the lung, bladder, and skin may be relatively different. These differences are particularly associated with the concentrations of oxygen, speciated arsenicals in the tissues, endogenous reducing agents, and ferritin. The urinary arsenic metabolic profiles are not necessarily reflective of the target tissue dosimetry because different tissues have different metabolite profiles. The direct and indirect roles of arsenic exposure in assisting carcinogenesis have been documented (29). High cumulative arsenic exposure from drinking water has been suggested to significantly increase the expression of plasma TGFα in a few cancer cases (32). Thus, in people exposed to InAs, the risk of cancers may be promoted through multiple biological trails, including the methylation capacity, which varies across populations.

We identified a dose–response relationship between InAs exposure and the risk of lung cancer among people with low methylation capacity. Notably, the risk of lung cancer was linearly proportional among those exposed to InAs from 2 ppb to >200 ppb, which may provide reference for future investigations on the mode of arsenic carcinogenesis among a particular high-risk population. In addition, the findings can aid future researches on preventive strategies and management according to the characteristics of susceptible population.

The strength of this study is the cohort design with clear temporality and low information bias. In addition, the sufficient length of observation (>40 years) and completeness of cancer registration system improved the validity of the findings. Although the findings are innovative and significant in the field of cancer research and arsenic carcinogenesis, this study has some limitations. First, a prospective design using repeated measurements of the participants' urinary InAs metabolite profiles is required for confirming the reliability of the dosimetry. Second, the samples were primarily from Chinese or Taiwanese populations. Although the results can be generalized globally, applications of these results to people of other ethnicities should be performed cautiously and should be affirmed by in-depth studies. Third, the InAs methylation pattern is a potential marker indicating an early detection of the high-risk group; however, further validation of the involved biological pathways by genomic or proteomic studies is required.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.-H. Hsu, K.-H. Tsui, H.-Y. Chiou, C.-J. Chen
Development of methodology: K.-H. Hsu, C.-J. Chen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.-H. Hsu, L.-I. Hsu, H.-Y. Chiou, C.-J. Chen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.-H. Hsu, L.-I. Hsu, C.-J. Chen

Writing, review, and/or revision of the manuscript: K.-H. Hsu, K.-H. Tsui, H.-Y. Chiou, C.-J. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.-H. Hsu, K.-H. Tsui, L.-I. Hsu
Study supervision: K.-H. Hsu, H.-Y. Chiou, C.-J. Chen

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