Carcinogenic Effects of “Whole-Life” Exposure to Inorganic Arsenic in CD1 Mice

Erik J. Tokar,* Bhalchandra A. Diwan,† Jerrold M. Ward,‡ Don A. Delker,§ and Michael P. Waalkes*,¹

*Laboratory of Comparative Carcinogenesis, National Cancer Institute at the National Institute of Environmental Health Sciences, and National Toxicology Program, The National Institute of Environmental Health Sciences, Research Triangle Park, Raleigh, North Carolina 27709; †Basic Research Program, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, Maryland 21773; ‡Global VetPathology, Montgomery Village, Maryland 20886; and §University of Utah, School of Medicine, 30 North 1900 East, Salt Lake City, Utah 84132

¹To whom correspondence should be addressed at National Toxicology Program, National Institute of Environmental Health Sciences, 111 Alexander Drive, PO Box 12233, MD F0-09, Research Triangle Park, NC 27709. Fax: (919) 541-3970. E-mail: waalkes@niehs.nih.gov.

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In a previously developed mouse model, arsenic exposure *in utero* induces tumors at multiple sites in the offspring as adults, often duplicating human targets. However, human environmental inorganic arsenic exposure occurs during the entire life span, not just part of gestation. Thus, “whole-life” inorganic arsenic carcinogenesis in mice was studied. CD1 mice were exposed to 0, 6, 12, or 24 ppm arsenic in the drinking water 2 weeks prior to breeding, during pregnancy, lactation, and after weaning through adulthood. Tumors were assessed in offspring until 2 years of age. Arsenic induced dose-related increases in lung adenocarcinoma (both sexes), hepatocellular carcinoma (both sexes), gallbladder tumors (males), and uterine carcinomas. Arsenic induced dose-related increases in ovarian tumors (including carcinomas) starting with the lowest dose. Adrenal tumors increased at all doses (both sexes). Arsenic-induced lung and liver cancers were highly enriched for cancer stem cells, consistent with prior work with skin cancers stimulated by prenatal arsenic. Reproductive tract tumors overexpressed cyclooxygenase-2 and estrogen receptor- α . Arsenic target sites were remarkably similar to prior transplacental studies, although tumors from whole-life exposure were generally more aggressive and frequent. This may indicate that arsenic-induced events *in utero* dictate target site in some tissues, whereas other exposure periods of arsenic enhance incidence or progression, though other factors could be at play, like cumulative dose. Whole-life arsenic exposure induced tumors at dramatically lower external doses than *in utero* arsenic only while more realistically duplicating human exposure.

Key Words: arsenic; carcinogenesis; mice; whole-life exposure.

Inorganic arsenic is a human carcinogen, and contaminated drinking water is a major route of exposure (IARC, 2004, 2009). Exposure to inorganic arsenic in drinking water is clearly linked to human lung, skin, and urinary bladder cancer and potentially linked to liver, prostate, and kidney cancer (IARC, 2004, 2009). Inorganic arsenic likely has multiple mechanisms of carcinogenic action (IARC, 2004).

Accumulating data suggest that early-life events can be critical in causing disease later in life (Barker, 2007). Conceptually developed from studies where early-life undernutrition precipitated adverse effects in adulthood (Barker, 2007), early-life toxicant exposure similarly can stimulate adulthood disease, including cancer (Anderson *et al.*, 2000; Birnbaum and Fenton, 2003; Waalkes *et al.*, 2007). For instance, growing evidence indicates that *in utero* or early-life inorganic arsenic exposure can cause human (Liaw *et al.*, 2008; Smith *et al.*, 2006; Yuan *et al.*, 2010) and rodent (Tokar *et al.*, 2010a; Waalkes *et al.*, 2003, 2004b, 2006a,b) cancer in adulthood. In humans, early-life arsenic exposure causes lung (Smith *et al.*, 2006), liver (Liaw *et al.*, 2008), and kidney (Yuan *et al.*, 2010) cancer. In mice, lung and liver cancers occur after *in utero* exposure to inorganic arsenic, whereas prenatal arsenic plus postnatal treatment with other tumor stimulants can drive the formation of skin and urinary bladder cancers (Waalkes *et al.*, 2003, 2004b, 2006a,b, 2008). The time difference between developmental exposure and adulthood cancer dictates that whatever the mechanism, it shows extended quiescence but then emerges later in life. Evolving theory indicates that cancers often arise in pluripotent stem cell (SC) populations, creating cancer SCs (CSCs) with highly distorted SC functions (Sullivan *et al.*, 2010; Wicha *et al.*, 2006). Normal SCs have key qualities required for tissue perpetuation and repair, like self-renewal, quiescence until needed, and conditional immortality (Sullivan *et al.*, 2010; Wicha *et al.*, 2006). These qualities, upon distortion by chemical insult, may provide a lifelong, latent CSC pool capable of subsequent tumor formation (Kangsamaksin *et al.*, 2007). Fetal SCs are probably key targets in transplacental carcinogenesis (Anderson *et al.*, 2000; Waalkes *et al.*, 2007), impacting fetal sensitivity to chemical carcinogenesis based, in part, on relative abundance. Indeed, *in utero* exposure to arsenic in Tg.AC mice results in marked stimulation of skin carcinoma formation in adulthood that shows a stunning CSC overabundance compared with

carcinoma formed without arsenic pretreatment (Waalkes *et al.*, 2008). Also, an arsenic-specific survival selection of SCs occurs during malignant transformation of human prostate epithelial cells compared with other carcinogens, causing a remarkable overabundance of CSCs only in the arsenic transformant (Tokar *et al.*, 2010c), consistent with the CSC overabundance in our skin cancer model (Waalkes *et al.*, 2008). The role of overproduction of CSCs in other tumors resulting from developmental arsenic exposure deserves study.

Gestation can often be a period of sensitivity to initiation of chemical carcinogenesis because of processes like organogenesis, global proliferative growth, and differentiation (Anderson *et al.*, 2000; Birnbaum and Fenton, 2003; Waalkes *et al.*, 2007). However, for environmental carcinogens, it is likely that humans would have full life exposure. Our transplacental studies use maternal oral treatment with inorganic arsenic (42.5 and 85 ppm) from gestation days 8 to 18, and although showing consistent carcinogenic activity in the offspring as adults (Tokar *et al.*, 2010a; Waalkes *et al.*, 2003, 2004b, 2006a,b), this protocol does not duplicate typical human exposure. Arsenic has a very short biological half-life (~5 days), and with these protocols, arsenic would be rapidly gone from the newborn. Furthermore, a complex arsenic adaptation occurs, involving metabolic and transporter changes (Coppin *et al.*, 2008), and how this protocol would impact this adaptation is unknown.

Thus, this study chronically exposed mice during their “whole life” to inorganic arsenic as would be expected in humans exposed to arsenic-contaminated drinking water. Others have noted the importance of whole-life treatments in rodent bioassays and proposed that these become a standard for carcinogenesis assessment in animals (Huff *et al.*, 2008). In the current whole-life inorganic arsenic protocol, breeder males and females were treated before breeding, the pregnant females were treated during the entire pregnancy, the dams were treated while lactating, offspring were treated for up to 2 years of age, and tumors were assessed in the offspring. Because the treatment was more protracted than prior transplacental studies, much lower doses of arsenic (6, 12, and 24 ppm arsenic) were used. These levels capture the upper range that has been reported for human drinking water exposure levels (IARC, 2004).

MATERIALS AND METHODS

Animals and treatment. Animal care was provided in accordance with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996). The CD1 mouse strain was used (Charles River Laboratories). Animals were treated humanely and with regard for alleviation of suffering, housed under conditions of controlled temperature, humidity, and light cycle, and provided a basal diet (5L79; Ralston Purina, St Louis, MO) and acidified water *ad libitum*. The NCI-Frederick animal facility, where the bioassay portion of the present study was conducted, and its animal program are accredited by the American Association for Accreditation of Laboratory Animal Care.

Sodium arsenite (NaAsO_2 , purity 98%) was obtained from Sigma Chemical Co. (St Louis, MO) and added to the drinking water in all cases. The levels used were 0 (control), 6, 12, and 24 ppm arsenic as sodium arsenite. Seven male and 14 female breeders were used for control and 6-ppm arsenic treatment groups, whereas 8 males and 16 females were used for 12- and 24-ppm arsenic treatment groups. Pregnant females received either normal drinking water (seven control females) or drinking water containing 6 ppm (seven females), 12 ppm (eight females), or 24 ppm arsenic (eight females). At birth, litters were culled to no more than eight. After birth, the dams received arsenic in the drinking water throughout lactation. At weaning (4 weeks of age), offspring were randomly selected from litters and divided into gender-based groups ($n = 30/\text{dose}$ for each sex) and continued to receive arsenic in the drinking water through adulthood to planned euthanasia at 104 weeks. This protocol will be henceforth termed whole-life arsenic exposure. Tumors were assessed only in the offspring.

For some immunohistochemical comparisons, samples of lung adenocarcinoma from adult BIO.A mice born to mothers that had received 0.5 mmol/kg of N-nitrosoethylurea (ENU), *sc*, on gestation day 16 (Rice *et al.*, 1987) were used. Archival paraffin blocks were employed for staining with CD133, a SC and CSC marker.

Clinical data and pathology. Maternal body weights were recorded during pregnancy. Individual body weights were recorded every 5 weeks after weaning. Only terminal body weights are reported for brevity. Clinical signs were checked daily. Mice were killed with CO_2 when moribund or at the study termination. A complete necropsy was performed on all moribund animals, animals found dead, or at the terminal sacrifice. The skin was carefully inspected for gross lesions. The following tissues were taken and processed for histological analysis: liver, kidneys (renal tubules), lungs, adrenals, spleen, thymus, brains, pituitary, gallbladder, urinary bladder, testes, ovary, uterus, oviduct, and all grossly abnormal tissues. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm , and stained with hematoxylin and eosin for histological analysis. The urinary bladder was inflated with fixative prior to being embedded. The pathologists were unaware of the treatment group during pathological assessments.

Immunohistopathology. Paraffin-embedded sections (5 μm) of liver tumors, lung tumors, uterine lesions, and ovarian tumors from both control and arsenic-treated mice were used for immunohistochemical analysis. Portions of lung and liver tumors from control and treated animals were compared for localization and intensity of aldehyde dehydrogenase-1 (ALDH1). Similarly, localization and intensity of estrogen receptor- α (ER- α), cyclin D1, cyclooxygenase-2 (Cox-2), and nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF- κB) were studied in uterine lesions and ovarian tumors. For this, polyclonal antibodies, ALDH1 (1:2000) and ER- α (1:1000; both from Santa Cruz Biotechnology, Santa Cruz, CA), cyclin D1 (1:1500; Upstate, NY), Cox-2 (1:1000; Cayman Chemical, Ann Arbor, MI), and CD133 (1:400) and NF- κB (1:100; both from Abcam, Cambridge, MA) were used. Diaminobenzidine from the Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) was used for final detection. To define specificity, the primary antibodies were omitted from each staining series as a control. All tissues were evaluated without prior knowledge of the treatment group.

Data analysis. Data are given as lesion incidence (number of affected mice/total mice available for examination) or as mean $\pm$ SEM, as appropriate. A probability level of $p \leq 0.05$ indicates a significant difference in all cases. In pairwise comparison of lesion incidence, a one-sided Fisher's exact test was used. To analyze dose-related trends in incidence, a two-sided Chi-square test for trend was used. For multiple comparisons of mean survival or body weight data, a two-sided Dunnett's test was used. Incidence is based on numbers of animals available for pathological analysis, and loss of animals to observation was typically because of postdeath autolysis considered too advanced for appropriate diagnosis. Sometimes tumors from a given tissue that are considered on a pathological continuum (adenoma and carcinoma) were combined for analysis, and in these cases if an animal had multiple tumors of different grade, it was considered as a single event in the higher grade group.

Similarly, the incidence of hyperplasia, adenoma, and carcinoma was sometimes combined as a pathological continuum and a mouse with multiple proliferative lesions was assigned to only the group dictated by the highest grade lesion. The combined incidence of hyperplasia, adenoma, and carcinoma in a given tissue was termed total proliferative lesions (TPL).

In one case, data for carcinoma incidence in adult female CD1 mice exposed to transplacental arsenic via the drinking maternal system (85 ppm in the drinking water) that had been previously reported (Waalkes *et al.*, 2006a) are rereported for comparison to whole-life data at the same target sites. The mice in both studies were obtained from the same supplier, housed in the same facility, and assessed by the same pathology team. Mice from the prior transplacental study (Waalkes *et al.*, 2006a) did, however, receive a different diet (National Institutes of Health Formula 31) than the CD1 mice in the present study. Data in female and male CD1 mice (run concurrently but reported separately; Waalkes *et al.*, 2006a,b) also provide some historical controls for spontaneously occurring tumors in control mice.

RESULTS

Survival and Body Weights

Maternal body weights during pregnancy were unaltered by treatment (not shown). Survival and body weight of the progeny mice were not altered by the whole-life arsenic exposure (Table 1). These data indicate that these levels of arsenic were well tolerated. Body weights are shown for 25, 50, and 104 weeks only but were similarly unaffected at other times during the bioassay.

Tumors and Proliferative Lesions in Male CD1 Mice

Whole-life arsenic exposure induced various tumors in male mice (Table 2). Liver hepatocellular carcinomas were increased at 12 and 24 ppm, and both liver carcinoma and total liver tumors (adenoma plus carcinoma) showed robust dose-response relationships. Hepatocellular carcinoma incidence approached significance ($p = 0.056$) above control in mice

even at 6 ppm arsenic. Male arsenic-exposed mice showed an approximately fourfold increase in lung adenocarcinoma at 24 ppm and a dose-response relationship for lung adenocarcinoma. The latter finding is blunted by the fact that the total incidence of lung tumors was, however, not increased in males, potentially indicating a shift from benign to malignant tumors with arsenic. Such a shift may account for the observed absence of a dose-response relationship in total lung tumors in male mice exposed to arsenic. Adrenal cortical adenoma occurred at all levels of arsenic exposure and showed a strong dose-response relationship.

Total gallbladder tumors (adenoma plus carcinoma) were increased at 24 ppm compared with control, and adenoma incidence approached significance ($p = 0.0518$) compared with control (0%). Gallbladder hyperplasia was increased at 6 and 12 ppm and approached a significant increase ($p = 0.0518$) at 24 ppm compared with control (0%). In fact, control animals showed no evidence of any proliferative lesions in the gallbladder. TPL (incidence of hyperplasia plus adenoma plus carcinoma, see “Materials and Methods” section) of the gallbladder were elevated at all levels of exposure in males and showed a dose-response relationship. Other organs in males showing proliferative lesions included the urinary bladder where hyperplasia was increased at all exposure levels of arsenic. The biomethylation product, dimethylarsinic acid, will also cause urinary bladder proliferative lesions in rats, including tumors (Arnold *et al.*, 2006). In the kidney, cystic hyperplasia of the renal tubules and TPL increased at all levels of exposure.

Tumors and Proliferative Lesions in Female CD1 Mice

Hepatocellular carcinomas and total liver tumors were increased at 24 ppm, and both showed dose-response relationships in female CD1 mice (Table 3). Lung adenocarcinomas and total lung tumors increased at both the 12 and 24 ppm.

TABLE 1
Survival and Body Weights in CD1 Mice after Whole-Life Inorganic Arsenic Exposure

			Survival			Body weight (g)		
Gender	Dose (ppm)	Initial group size	Average (weeks)	Mice at 52 weeks	Mice at 104 weeks	Mice at 25 weeks	Mice at 50 weeks	Mice at 104 weeks
Females								
	0	30	80.3 ± 4.5	25 (83%)	12 (40%)	42.4 ± 1.0	48.4 ± 1.3	46.2 ± 1.3
	6	30	82.4 ± 3.8	26 (87%)	7 (23%)	45.3 ± 1.7	51.8 ± 2.4	49.8 ± 4.5
	12	30	81.5 ± 5.0	22 (73%)	10 (33%)	38.2 ± 1.0	44.4 ± 1.7	43.2 ± 3.1
	24	30	89.2 ± 3.4	28 (93%)	13 (43%)	38.7 ± 1.4	46.5 ± 2.0	39.6 ± 2.2
Males								
	0	30	85.0 ± 4.8	23 (76%)	12 (40%)	53.4 ± 1.4	59.6 ± 1.8	52.5 ± 2.7
	6	30	79.4 ± 4.0	26 (87%)	9 (30%)	56.2 ± 1.3	61.6 ± 1.6	55.8 ± 2.0
	12	30	87.5 ± 3.9	28 (93%)	12 (40%)	50.2 ± 1.0	55.8 ± 1.3	48.6 ± 2.3
	24	30	88.7 ± 2.7	28 (93%)	9 (30%)	52.9 ± 1.1	56.2 ± 1.3	50.9 ± 1.2

Arsenic was given continuously at the level indicated as sodium arsenite to the breeding pairs prior to breeding for 2 weeks, to pregnant dams, to lactating dams, and then to the offspring from weaning up to 2 years of age when the experiment ended (see “Materials and Methods” section). Tumors were only assessed in the offspring. Data are expressed as the mean ± SEM or number of animals surviving at a given time (percentage of total animals on test). No significant differences from gender-matched control occurred in survival in average weeks, in survival percent at a given time point, or in body weight at 25, 50, or 104 weeks ($p > 0.05$).

TABLE 2
Tumors and Proliferative Lesions Induced by Whole-Life
Arsenic Exposure in Male CD1 Mice

Tissue Lesion (%)	Group (n)				Trend <i>p</i>
	0 ppm (29)	6 ppm (29)	12 ppm (28)	24 ppm (28)	
Liver					
Adenoma	2 (7%)	3 (10%)	3 (11%)	6 (21%)	0.111
Carcinoma	0 (0%)	4 (14%) ^a	6 (21%)*	6 (21%)*	0.013*
Total	2 (7%)	6 (21%)	7 (25%)	10 (36%)*	0.009*
Gallbladder					
Hyperplasia	0 (0%)	5 (17%)*	5 (18%)*	4 (14%) ^b	0.213
Adenoma	0 (0%)	2 (7%)	3 (11%)	4 (14%) ^b	0.039*
Carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0.173
Total tumors	0 (0%)	2 (7%)	3 (11%)	5 (18%)*	0.016*
TPL ^c	0 (0%)	7 (24%)*	8 (29%)*	11 (39%)*	0.014*
Lung					
Adenoma	7 (24%)	3 (10%)	5 (18%)	2 (7%)	0.145
Adenocarcinoma	3 (10%)	8 (28%)	9 (32%)	11 (39%)*	0.014*
Total	10 (34%)	11 (38%)	11 (39%)	13 (46%)	—
Adrenal adenoma	0 (0%)	7 (24%)*	7 (25%)*	9 (32%)*	0.014*
UB hyperplasia ^d	0 (0%)	6 (21%)*	7 (24%)*	6 (21%)*	0.027*
Kidney					
Hyperplasia	1 (3%)	7 (24%)*	7 (25%)*	6 (21%)*	0.115
Adenoma	1 (3%)	0 (0%)	2 (7%)	0 (0%)	—
Carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	—
TPL ^c	2 (7%)	7 (24%)*	8 (29%)*	7 (24%)*	0.040*

Arsenic was given continuously throughout life (see “Materials and Methods” section for details). Sample size (*n*) is the number of mice available for pathological analysis. Data are expressed as mice with a given lesion (percent undergoing assessment). Total tumors do not always equal the sum of adenoma plus carcinoma because multiple tumors of different grades occurred in the same mouse.

^aApproached statistical significance from control (*p* = 0.0560).

^bApproached statistical significance from control (*p* = 0.0518).

^cIncludes mice with at least one hyperplasia, adenoma, or carcinoma of the tissue in question.

^dUB, urinary bladder; one papilloma occurred in a mouse exposed to 6 ppm arsenic (not included in hyperplasias).

*Significant from control or a significant dose-related trend (*p* ≤ 0.05).

Both lung adenocarcinoma incidence and total lung tumor incidence showed robust dose-response relationships. Gallbladder lesions were erratic and only occurred to a significant level at 12 ppm when considering TPL, primarily because of early lesions (hyperplasia). Adrenal cortical adenoma occurred in all arsenic-treated female mice and showed a dose-response. Urinary bladder hyperplasia increased significantly only at 6 ppm, whereas kidney proliferative lesions were not elevated. The reasons for the lack of dose-response relationship in gallbladder and urinary bladder proliferative lesions in females are not immediately apparent.

Several tumors and proliferative lesions occurred in the female reproductive tract with whole-life arsenic exposure. Total ovarian tumors were increased at all exposure levels and showed a significant dose-response. Ovarian adenoma incidence was increased at 24 ppm, approached significance at

TABLE 3
Tumors and Proliferative Lesions Induced by Whole-Life
Arsenic Exposure in Female CD1 Mice

Tissue Lesion (%)	Group (n)				Trend <i>p</i>
	0 ppm (29)	6 ppm (29)	12 ppm (28)	24 ppm (28)	
Liver					
Adenoma	1 (3%)	1 (3%)	2 (7%)	1 (4%)	—
Carcinoma	0 (0%)	2 (7%)	2 (7%)	5 (18%)*	0.018*
Total	1 (3%)	3 (10%)	4 (14%)	6 (21%)*	0.011*
Gallbladder					
Hyperplasia	0 (0%)	1 (4%)	4 (14%) ^a	0 (0%)	—
Adenoma	0 (0%)	0 (0%)	1 (4%)	0 (0%)	—
Carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	—
Total tumors	0 (0%)	0 (0%)	1 (0%)	0 (0%)	—
TPL ^b	0 (0%)	1 (4%)	5 (17%)*	0 (0%)	—
Lung					
Adenoma	4 (14%)	4 (14%)	6 (21%)	2 (7%)	—
Adenocarcinoma	2 (7%)	6 (21%)	8 (28%)*	12 (43%)*	0.001*
Total	6 (21%)	7 (24%)	14 (48%)*	14 (50%)*	0.005*
Adrenal adenoma	0 (0%)	5 (17%)*	7 (24%)*	7 (25%)*	0.008*
UB hyperplasia ^c	0 (0%)	6 (21%)*	2 (7%)	2 (7%)	—
Kidney					
Hyperplasia	0 (0%)	0 (0%)	1 (4%)	0 (0%)	—
Adenoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	—
Ovary					
Adenoma	0 (0%)	3 (10%)	4 (14%) ^a	5 (17%)*	0.026*
Carcinoma	0 (0%)	2 (7%)	2 (7%)	4 (14%) ^d	0.045*
Total	0 (0%)	5 (18%)*	6 (14%)* ^e	9 (21%)*	0.001*
Uterus					
Hyperplasia	3 (10%)	4 (14%)	11 (38%)*	6 (21%)	0.043*
Adenoma	1 (3%)	4 (14%)	3 (10%)	3 (10%)	0.452
Adenocarcinoma	0 (0%)	1 (3%)	3 (10%)	6 (21%)*	0.003*
Total tumors	1 (3%)	5 (17%)	6 (21%)	9 (32%)*	0.006*
TPL ^b	4 (14%)	8 (28%)	14 (48%)*	15 (54%)*	0.0005*
Oviduct					
Hyperplasia	1 (3%)	7 (24%)*	13 (45%)*	11 (38%)	0.0006*
Adenoma	0 (0%)	0 (0%)	2 (7%)	0 (0%)	—

Arsenic was given continuously throughout life (see “Materials and Methods” section for details). Sample size (*n*) is the number of mice available for pathological analysis. Data are expressed as mice with a given lesion (percent undergoing assessment). Total tumors do not always equal the sum of adenoma plus carcinoma because multiple tumors of different grades occurred in the same mouse.

^aApproached statistical significance from control (*p* = 0.0560).

^bIncludes mice with at least one hyperplasia, adenoma, or carcinoma of the tissue in question.

^cUB, urinary bladder.

^dApproached statistical significance from control (*p* = 0.0518).

^eA luteoma and a malignant thecoma occurred in 12 ppm arsenic-treated mice that had no other ovarian tumors but are not included in the total tumor number.

*Significant from control or a significant dose-related trend (*p* ≤ 0.05).

12 ppm (*p* = 0.056), and showed a dose-response relationship. Similarly, ovarian carcinoma incidence approached statistical significance at 24 ppm (*p* = 0.0518) and showed a significant dose-response relationship. Uterine adenocarcinoma and total uterine tumor incidence increased at 24 ppm, and both metrics

displayed robust dose-response relationships. Uterine TPL incidence increased to levels of 3.4- to 3.9-fold above control with 12 and 24 ppm, respectively, and showed a robust relationship to arsenic dose. Uterine hyperplasia also showed a dose-response relationship to whole-life arsenic exposure level. Oviduct hyperplasia was increased at all levels of whole-life arsenic exposure. An occasional oviduct adenoma occurred in the group exposed to 12 ppm arsenic. Oviduct hyperplasia appeared to reach a maximum incidence between 38 and 45%.

Evidence of CSC Overabundance in Tumors Induced by Whole-Life Arsenic Exposure

Prenatal arsenic exposure causes an overabundance of CSCs in skin squamous cell carcinomas in mice in adulthood (Waalkes *et al.*, 2008), whereas *in vitro* work has shown an arsenic-specific CSC overabundance during malignant transformation in human cells when compared with other carcinogens (Tokar *et al.*, 2010c). Thus, the relative abundance of CSCs in tumors induced by whole-life arsenic exposure was examined (Fig. 1). ALDH1 is a marker for liver (Ma *et al.*, 2008) and lung (Sullivan *et al.*, 2010) CSCs. There was little presence of ALDH1 protein in spontaneous liver tumors (see Fig. 1A), whereas hepatocellular carcinomas resulting from whole-life arsenic exposure showed strong, widespread presence of ALDH1 (e.g., see Fig. 1B). Similarly, lung adenocarcinomas from control female mice showed minimal ALDH1 protein, although it was widespread and often intense in arsenic-induced pulmonary adenocarcinoma (Figs. 1C and 1D, respectively). Importantly, as expected, ALDH1 protein was not present in the nuclei of cells enriched in this protein from arsenic-induced liver or lung tumors. The liver and lung CSC marker CD133 (Bertolini *et al.*, 2009; Rountree *et al.*, 2009) followed a similar pattern of elevation in arsenic-induced lung adenocarcinoma (Figs. 1E and 1F). A surface marker related to CSC phenotype because of its role in attachment of SCs to their niche (Bomken *et al.*, 2010), CD133, was highly elevated and widespread in lung adenocarcinoma from arsenic-treated animals, whereas lung adenocarcinoma resulting from prenatal ENU treatment showed only occasional CD133-positive cells, which would be expected if a CSC was relatively rare cell.

Comparative Effect of Whole-Life and Transplacental-Only Arsenic Exposures

Prior 2-year studies from our group used *in utero* arsenic exposure only in various strains of mice (Waalkes *et al.*, 2003, 2004b, 2006a,b), including CD1 mice (Waalkes *et al.*, 2006a,b), allowing a general comparison to the present work. In the prior transplacental work, pregnant mice were treated with up to 85 ppm arsenic from gestation days 8 to 18 and tumors were assessed in the offspring as adults (Waalkes *et al.*, 2003, 2004b, 2006a,b). Comparing these historical

FIG. 1. Overexpression of ALDH1 and CD133 in whole-life arsenic-exposed liver and lung tumors. ALDH1 is a common CSC marker in both liver and lung tumors. Spontaneous liver (A) and lung (C) tumors from control mice were compared with whole-life arsenic-induced liver (B) and lung (D) tumors. Immunohistological staining shows strong, widespread nonnuclear expression (brown color) of ALDH1 protein in arsenic-exposed sections compared with little presence of protein in spontaneous tumors in control sections. CD133 staining in ENU-induced (E) and whole-life arsenic-induced (F) lung tumors showing sporadic staining in the ENU tumor and widespread staining in the arsenic tumor. Results are typical from multiple ($n > 5$) different tumors from control of arsenic-treated mice. Scale bars = 100 μ m.

transplacental results to the present whole-life data indicated that both arsenic exposure protocols had the capacity to produce tumors in a remarkably similar series of target tissues, including the lung, liver, uterus, and ovary (present study Tables 2 and 3; Waalkes *et al.*, 2003, 2004b, 2006a,b), although with some gender and strain dependence. This indicates that *in utero* arsenic exposure may dictate tissue target site for some tissues. Second, a much lower exposure level in the whole-life protocol (24 ppm) produced a higher incidence of higher grade tumors, as noted by specifically comparing, e.g., results on carcinoma incidence in female CD1 mice with these target tissues from the present study and a study (Waalkes *et al.*, 2006a) run with *in utero* only exposure (85 ppm) alone in female CD1 mice (Fig. 2). For instance, after whole-life arsenic exposure compared with *in utero* exposure only, lung adenocarcinoma were increased nearly threefold, hepatocellular carcinoma were increased approximately six-fold, uterine adenocarcinoma were increased 3.5-fold, and ovarian carcinoma, which did not occur with *in utero* arsenic

FIG. 2. Carcinoma incidence in female mice following whole-life exposure (24 ppm) or transplacental-only exposure (85 ppm; gestation days 8–18) to inorganic arsenic. Compared with transplacental-only exposure, whole-life arsenic exposure generally increased carcinoma incidence. An asterisk (*) indicates significant increase ($p \leq 0.05$) over group-specific control (transplacental-only exposure or whole-life exposure), whereas a dagger (†) indicates a significant difference of whole-life compared with transplacental-only exposure. Transplacental data were previously reported (Waalkes *et al.*, 2006b) and are rereported here for comparative purposes. Ovarian carcinoma incidence in whole-life mice approached significance ($p = 0.0518$) over control (see Table 3). Historical control rates of these carcinoma (Waalkes *et al.*, 2006) were not statistically different from the control rates in the present work.

alone, increased dramatically (Fig. 2). Historical controls (Waalkes *et al.*, 2006a) for CD1 female mice are included for these sites and are unremarkable when compared with the controls of the present work.

Arsenic-Induced Uterine Tumors and Estrogen-Responsive Genes

Many sites in the female mouse reproductive tract (ovary, uterus, oviduct, etc.) can be impacted oncogenically by *in utero* arsenic exposure (Waalkes *et al.*, 2004a,b, 2006a,b) and by whole-life arsenic exposure in the present work (see Table 3). We find that *in utero* arsenic exposure results in upregulated ER- α in hepatocellular carcinoma from male mice (Waalkes *et al.*, 2004a), which likely contributes to carcinogenic activity in liver (Waalkes *et al.*, 2006b). In addition, humans living in areas where high environmental arsenic exposure is endemic and having skin lesions typical for chronic high arsenic intake show marked overexpression of hepatic ER- α (Waalkes *et al.*, 2004a). Thus, potential activation of ER- α in a representative tumor (uterine adenocarcinoma) of the female reproductive tract was tested. ER- α was highly upregulated in uterine adenocarcinoma from a whole-life arsenic-exposed mouse (Fig. 3A). ER- α was widespread, intense, and nuclear. Control uterine lesions showed much less evidence of ER- α protein (Fig. 3B). Estrogen also regulates cyclin D1 (Seth *et al.*, 2002), and cyclin D1 protein was greatly increased by whole-life arsenic exposure in uterine adenocarcinoma (Fig. 3C) compared with control (Fig. 3D). In addition, evidence indicates that NF- κ B overexpression with ER- α positivity together identify a high-risk subset of aggressive female endocrine-based tumors (Zhou *et al.*, 2005), and whole-life arsenic clearly activated NF- κ B overproduction (Fig. 3E) compared with control (Fig. 3F). The presence of Cox-2, which can be upregulated by arsenic through NF- κ B in a fashion relevant to the oncogenic process (Ouyang *et al.*, 2007), was clearly increased in uterine adenocarcinoma induced by whole-life arsenic exposure compared with control tissue (Figs. 3G and 3H, respectively). ER- α , cyclin D1, NF- κ B, and Cox-2 were all similarly increased in ovarian tumors induced by whole-life arsenic exposure (not shown). Thus, a cascade of genes that may be relevant to the carcinogenic process is upregulated in the female reproductive tract tumors by whole-life arsenic exposure.

Spontaneous Tumors Not Related to Treatment

Spontaneous tumors occurred that were not related to treatment. In male mice, this included the following: three liver hemangiomas, two lymphomas, a pancreatic islet cell carcinoma, a peritoneal fibrosarcoma, and a spleen histiocytic sarcoma in control; three liver hemangiomas, one liver hemangiosarcoma, and one spleen hemangioma at 6 ppm arsenic; four lymphomas at 12 ppm arsenic; and two liver hemangiosarcoma, one sc sarcoma, one basal cell carcinoma,

FIG. 3. Overexpression of estrogen-regulated markers in whole-life arsenic-induced uterine tumors. Immunohistological staining in whole-life arsenic-induced (left column) and spontaneous tumors in control mice (right column) for ER- α (A and B), cyclin D1 (C and D), NF- κ B (E and F), and Cox-2 (G and H) show that protein expression (brown color) of all estrogen-regulated markers in whole-life arsenic-induced tumors was much more intense and widespread when compared with spontaneous tumors from control mice. Results are typical from lesions from multiple different control or treated mice. Scale bars = 100 μ m.

one lymphoma, and one leukemia at 24 ppm arsenic. In female mice, this included the following: a liver hemangioma, a spleen hemangioma, a uterine sarcoma, nine lymphoma, and two leukemias in control; a liver liposarcoma, two uterine leiomyosarcomas, a stomach carcinoid tumor, a mammary

adenocarcinoma, and four lymphoma at 6 ppm arsenic; a uterine leiomyoma, a mammary adenocarcinoma, 10 lymphoma, and a leukemia at 12 ppm arsenic; and a liver hemangiosarcoma, a uterine sarcoma, a uterine leiomyoma, a lipoma, four lymphoma, a leukemia at 24 ppm arsenic.

DISCUSSION

Whole-life arsenic exposure in mice induced tumor formation in multiple tissues at levels as low as 6 ppm arsenic, within the upper range reported for human drinking water exposure (IARC, 2004). Combined with prior work, these data provide important insight into arsenic carcinogenesis. A key finding is that the target tissues of whole-life arsenic carcinogenesis in this work with CD1 mice (lung, liver, uterus, ovary, and adrenal), with a single addition (gallbladder), are identical to those induced in one or more of our prior transplacental arsenic exposure-alone studies using three mouse strains (CD1, C3H, and Tg.AC; Tokar *et al.*, 2010a; Waalkes *et al.*, 2003, 2004b, 2006a,b). The tumor distribution depends on gender, but the patterns are clear. Preneoplastic lesions of the kidney and urinary bladder can also occur after transplacental arsenic alone (Tokar *et al.*, 2010a; Waalkes *et al.*, 2006b), consistent with whole-life exposure. Together these data indicate that the early-life arsenic exposure may dictate target site for arsenic oncogenesis, whereas other periods in whole-life exposure could enhance tumor production, although alternative hypotheses need to be considered. The present data further fortify this contention, as whole-life arsenic at much lower external exposure doses induced a greater incidence of tumors than transplacental arsenic exposure alone. The concept that developmental exposure to a carcinogen dictates cancer target site is a corollary to the nutritional concept of developmental basis of adult disease (Barker, 2007) but applied to carcinogenesis. This implies that permanent, agent-driven oncogenic changes occurring during development direct cancer much later in life. This potential is highlighted in the emerging data showing that early-life exposure to inorganic arsenic from the drinking water is now linked to adulthood liver, lung, and kidney cancer in humans (Liaw *et al.*, 2008; Smith *et al.*, 2006; Yuan *et al.*, 2010). These probable or possible human targets of inorganic arsenic (IARC, 2004, 2009) can be duplicated in transplacental or whole-life exposure mouse models as neoplasia or preneoplastic lesions (Waalkes *et al.*, 2003, 2004b, 2006b; present study). That cancer “dictating” events occur during developmental exposure to inorganic arsenic may have broad implications concerning the notion of chemically induced developmental basis of adult disease after environmental exposure.

Although it may be attractive to hypothesize that *in utero* events may dictate adulthood tumor target site with inorganic arsenic, other possibilities exist, such as greater accumulated dose with whole-life arsenic exposure stimulating similar targets. A key control is missing in the present work that would validate an *in utero* target site dictation hypothesis. An adult inorganic arsenic exposure-only group with CD1 mice would be required to establish this fetal dictation hypothesis. In place of this, we have a long history of oral inorganic arsenical exposure studies in adult rodents that were negative for carcinogenicity. For example, various early studies fed or gave

in the drinking water levels of arsenic ranging from 5 to 250 ppm to mice or rats continuously over their adult lives and found no tumor response (Byron *et al.*, 1967; Kanisawa and Schroeder, 1967, 1969). These studies and others prompted the International Agency for Research on Cancer in 1980 to say that there was inadequate evidence of rodent carcinogenicity of arsenic despite unequivocal human evidence (IARC, 1980). Even in the 2004 evaluation (IARC, 2004), there was still considered limited evidence of inorganic arsenic carcinogenesis in rodents, and in 2005, a National Science Foundation panel stated that “arsenic does not cause cancer in laboratory animals” (NSF, 2005). An accumulated dose causation concept would assume that target site remained similar regardless of age, but additional arsenic treatment would be required in less sensitive periods, presumably including adulthood. Indeed, in this regard, highly sensitive adult A/J mice will show increased lung tumor multiplicity after chronic exposure to arsenic as adults (Cui *et al.*, 2006). Nonetheless, the notion that the current whole-life protocol used is active because of greater accumulated dose should be placed in perspective with the multiple attempts that have used similar or higher doses with chronic rodent adult-only inorganic arsenic exposure and found no tumor response with animals of usual sensitivity. Although not a perfect control, such studies represent 50 years of unsuccessful attempts to show inorganic arsenic to be carcinogenic in adult rodents (Byron *et al.*, 1967; IARC, 1973, 1980, 1987, 2004; Kanisawa Schroeder, 1967, 1969; NSF, 2005). However, only a truly controlled experiment that has an adult CD1 mouse exposure-only group will allow us to establish the role of *in utero* exposure in adulthood oncogenesis in this mouse model, and one is planned.

An apparent overabundance of CSCs was observed in liver and lung tumors induced after whole-life arsenic exposure. Inorganic arsenic can also induce malignant transformation of human prostate SCs *in vitro* that when inoculated into mice very rapidly produce highly pluripotent, very aggressive malignant tumors (Tokar *et al.*, 2010b). Prostate is considered a possible target of arsenic in humans (IARC, 2009). Additionally, arsenic-induced malignant transformation in a mature prostate epithelial cell line appears dictated by a SC survival selection that results in a stunning CSC overabundance during transformation that is specific to arsenic, as transformation of the same cell line with cadmium or methylnitrosourea, although causing CSC formation, does not induce CSC overabundance (Tokar *et al.*, 2010c). In a human skin cancer model, arsenic slows SC differentiation and increases the proportion of the SCs, likely key targets for carcinogenesis (Patterson *et al.*, 2005; Patterson and Rice, 2007). These data are consistent with our skin cancer model in Tg.AC mice where transplacental arsenic exposure causes a hypersensitivity to skin carcinogenesis in adulthood and a proclivity toward more advanced and aggressive skin tumors with additional treatment with a remarkable CSC overabundance compared with tumors formed without prenatal arsenic pretreatment (Waalkes *et al.*,

2008). Thus, the apparent CSC overabundance in lung and liver malignancies after whole-life arsenic exposure in the present work is consistent with prior work *in vivo* (Waalkes *et al.*, 2008) and *in vitro* (Patterson *et al.*, 2005; Patterson and Rice, 2007; Tokar *et al.*, 2010b,c). Developing theory predicts that many tumors arise in pluripotent SC/progenitor cell populations (Sullivan *et al.*, 2010; Wicha *et al.*, 2006) because the SC qualities of self-renewal, conditional immortality, and quiescence until required have the potential to provide for a lifelong occult neoplastic cell population once altered by a chemical carcinogen (Kangsamaksin *et al.*, 2007). Clearly, development is a time of greater sensitivity to chemical carcinogens (Anderson *et al.*, 2000; Birnbaum and Fenton, 2003; Waalkes *et al.*, 2007), and SCs in the developing animal are probably key targets for this sensitivity (Anderson *et al.*, 2000; Waalkes *et al.*, 2007) based on relative abundance, rapid growth, differentiation, etc. Accepting cancer as a disease driven by SC dysregulation (Wicha *et al.*, 2006), liver and lung tumor CSC overabundance resulting from whole-life arsenic exposure is consistent with both *in vivo* and *in vitro* models where arsenic causes aberrant accumulation of SCs or CSCs (Patterson *et al.*, 2005; Patterson and Rice, 2007; Tokar *et al.*, 2010b,c; Waalkes *et al.*, 2008), and together these data are consistent as a key component of a potential mode of action.

Inorganic arsenic has both carcinogenic (IARC, 2004, 2009) and antitumor potential (Kim *et al.*, 2010; Soignet *et al.*, 1998). In a recent study, 85 ppm arsenic given from gestation day 8 to 1 year of postnatal age suppressed spontaneous liver tumors in male C3H mice (Nelson *et al.*, 2009), a strain with a high spontaneous rate. This same dose of arsenic in the maternal drinking water given only during gestation (days 8–18) to C3H mice consistently increases liver tumors over control when observed for 2 years (Waalkes *et al.*, 2003, 2004b). Whole-life arsenic exposure increased liver cancers at much lower doses in both male and female CD1 mice over 2 years. Because many mouse liver tumors occur after 1 year, the suppression of liver tumors at 1 year is somewhat difficult to interpret. It is possible that this higher dose of arsenic acted as a cancer initiator and then subsequently acted as a chemotherapeutic for the very cancers it initiated. In fact, inorganic arsenic has undergone human trial for use against hepatocellular carcinoma (Chen *et al.*, 2002), and with it blocking certain signaling pathways, there is the enthusiasm that this may be extended to other solid tumors (Kim *et al.*, 2010). Although, this should be done with care because of the risk of secondary tumor formation. However, this serves to point out the critical importance for movement toward human environmental exposure levels and whole-life exposure in carcinogen testing in rodents with arsenic and other agents of environmental concern.

Consistent with our previous transplacental arsenic exposure studies (Waalkes *et al.*, 2004a, 2006b), whole-life arsenic exposure also upregulates ER- α and ER- α -associated genes like NF- κ B, Cox-2, and cyclin D1. ER- α is also upregulated in liver from men with dermal lesions typical of chronic

arsenicalism living in areas where high environmental arsenic exposure is common (Waalkes *et al.*, 2004a), suggesting that this ER- α upregulation also occurs with chronic arsenic exposure in human populations. ER signaling can occur through the mitogen-activated protein kinase and the phosphoinositide-3-kinase pathways leading to ligand-dependent and independent ER-mediated gene activation (Zhou *et al.*, 2005). NF- κ B plays a critical role in normal organ development, can induce expression of several antiapoptotic genes, and promote proliferation, invasion, and motility (Cao and Karin, 2003). Most interaction between ER- α and NF- κ B is in a functional antagonistic manner, but these two factors can also act synergistically at the transcriptional and nongenomic levels. For instance, ER- α and NF- κ B can act synergistically through a nongenomic mechanism to activate the NF- κ B-dependent gene product Cox-2 (Pedram *et al.*, 2002). Arsenic exposure can specifically increase Cox-2 expression through a NF- κ B pathway, contributing to arsenic-induced carcinogenesis likely through an antiapoptotic process (Ouyang *et al.*, 2007). This would help to explain the marked increase in Cox-2 expression in the uterine tumors from arsenic-exposed mice. Moreover, positive cross talk between ER- α and NF- κ B has been associated with a more aggressive phenotype (Frasor *et al.*, 2009) and with the identification of a high-risk subset of ER- α -positive breast cancers (Zhou *et al.*, 2005). Cyclin D1 is a cell cycle regulator and oncogene linked with poor prognosis in ER-positive cancers (Yang *et al.*, 2010) and can be upregulated by both ER- α and NF- κ B (De Bosscher *et al.*, 2006). Thus, it appears that arsenic can upregulate ER- α , NF- κ B, and ER- α -associated factors through transcriptional and nongenomic pathways, suggesting multiple potential mechanisms for carcinogenic activity during whole-life arsenic exposure.

In this study, the gallbladder was a target for whole-life arsenic exposure in males. Gallbladder cancers are considered by many as rare tumors in humans (Wistuba and Gazdar, 2004) and have not been clearly associated with arsenic exposure. Rodents treated orally with inorganic arsenic will excrete arsenic glutathione complexes into the bile, some of which can decompose into methylarsonous acid (MMA^{III}; Kobayashi and Hirano, 2008) considered one of the most toxic and possibly the most carcinogenic of the arsenical biomethylation species. With whole-life arsenic exposure, it is possible that the gallbladder was repeatedly exposed to MMA^{III} and this led to tumor formation, although the reasons for gender disparity would be unclear. Further study is required to define the role of metabolism and gender in arsenic-induced gallbladder cancer.

In summary, whole-life inorganic arsenic exposure was an effective carcinogen in multiple tissues in mice at exposure levels approaching those to which humans can be exposed, producing tumors in the same target sites as our prior work using *in utero* exposure alone but producing more tumors at lower exposure levels of a more advanced nature. This

indicates that developmental arsenic exposure may dictate target site, whereas other periods of exposure could act to enhance the carcinogenic response. Accumulating data showing that inorganic arsenic exposure during human development is carcinogenic in adulthood fortify the importance of these present data in mice.

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REFERENCES

- Anderson, L. M., Diwan, B. A., Fear, N. T., and Roman, E. (2000). Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. *Environ. Health Perspect.* **108**, 573–594.
- Arnold, L. L., Eldan, M., Nyska, A., van Gemert, M., and Cohen, S. M. (2006). Dimethylarsinic acid: results of chronic toxicity/oncogenicity studies in F344 rats and in B6C3F1 mice. *Toxicology* **223**, 82–100.
- Barker, D. J. P. (2007). The origins of the developmental origins theory. *J. Intern. Med.* **261**, 412–417.
- Bertolini, G., Roz, L., Perego, P., Tortoreto, M., Fontanella, E., Gatti, L., Pratesi, G., Fabbri, A., Andriani, F., Tinelli, S., et al. (2009). Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16281–16286.
- Birnbaum, L. S., and Fenton, S. E. (2003). Cancer and developmental exposure to endocrine disruptors. *Environ. Health Perspect.* **111**, 389–394.
- Bomken, S., Fiser, K., Heidenreich, O., and Vormoor, J. (2010). Understanding the cancer stem cell. *Br. J. Cancer* **103**, 439–445.
- Byron, W. R., Bierbower, G. W., Brouwer, J. B., and Hansen, W. H. (1967). Pathologic changes in rats and dogs from two-year feeding of sodium arsenite or sodium arsenate. *Toxicol. Appl. Pharmacol.* **10**, 132–147.
- Cao, Y., and Karin, M. (2003). NF-kappaB in mammary gland development and breast cancer. *J. Mammary Gland Biol. Neoplasia* **8**, 214–223.
- Chen, Z., Chen, G. Q., Shen, Z. X., Sun, G. L., Tong, J. H., Wang, Z. Y., and Chen, S. J. (2002). Expanding the use of arsenic trioxide: leukemias and beyond. *Semin. Hematol.* **39**, 22–26.
- Coppin, J., Qu, W., and Waalkes, M. P. (2008). Interplay between cellular methyl metabolism and adaptive efflux during oncogenic transformation from chronic arsenic exposure in human cells. *J. Biol. Chem.* **283**, 19342–19350.
- Cui, X., Wakai, T., Shirai, Y., Hatakeyama, K., and Hirano, S. (2006). Chronic oral exposure to inorganic arsenate interferes with methylation status of p16INK4a and RASSF1A and induces lung cancer in A/J mice. *Toxicol. Sci.* **91**, 372–381.
- De Bosscher, K., Venden Berghe, W., and Haegeman, G. (2006). Cross-talk between nuclear receptors and nuclear factor kB. *Oncogene* **25**, 6868–6886.
- Frasor, J., Weaver, A., Pradham, M., Dai, Y., Miller, L. D., Lin, C.-Y., and Stanculescu, A. (2009). Positive cross-talk between estrogen receptor and NH-kB in breast cancer. *Cancer Res.* **69**, 8918–8925.
- Huff, J., Jacobson, M. F., and Davis, D. L. (2008). The limits of two-year bioassay exposure regimens for identifying chemical carcinogens. *Environ. Health Perspect.* **116**, 1439–1442.
- Institute of Laboratory Animal Resources (ILAR). (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, DC.
- International Agency for Research on Cancer (IARC). (1973). *International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Inorganic and Organometallic Compounds, Arsenic and Inorganic Arsenic Compounds*, Vol. 2, pp. 48–73. IARC Press, Lyon, France.
- International Agency for Research on Cancer (IARC). (1980). *International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Metals and Metallic Compounds, Arsenic and Arsenic Compounds*, Vol. 23, pp. 39–143. IARC Press, Lyon, France.
- International Agency for Research on Cancer (IARC). (1987). *International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs. Arsenic and Arsenic Compounds*, Vol. 1–42, pp. 100–106. IARC Press, Lyon, France.
- International Agency for Research on Cancer (IARC). (2004). *Arsenic in Drinking Water. International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risk to Humans. Some Drinking Water Disinfectants and Contaminants, Including Arsenic*, Vol. 84, pp. 269–477. IARC Press, Lyon, France.
- International Agency for Research on Cancer (IARC). (2009). *IARC Special Report: Policy, A review of Human Carcinogens—Part C: Metals, Arsenic, Dusts, and Fibres*, Vol. 10, pp. 453–454. IARC Press, Lyon, France.
- Kangsamaksin, T., Park, H. J., Trempus, C. S., and Morris, R. J. (2007). A perspective on murine keratinocyte stem cells as targets of chemically induced skin cancer. *Mol. Carcinog.* **46**, 579–584.
- Kanisawa, M., and Schroeder, H. A. (1967). Life term studies on the effects of arsenic, germanium, tin, and vanadium on spontaneous tumors in mice. *Cancer Res.* **27**, 1192–1195.
- Kanisawa, M., and Schroeder, H. A. (1969). Life term studies on the effect of trace elements on spontaneous tumors in mice and rats. *Cancer Res.* **29**, 892–895.
- Kim, J., Lee, J. J., Kim, J., Gardner, D., and Beachy, P. A. (2010). Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13432–13437.

- Kobayashi, Y., and Hirano, S. (2008). Effects of endogenous hydrogen peroxide and glutathione on the stability of arsenic metabolites in rat bile. *Toxicol. Appl. Pharmacol.* **232**, 33–40.
- Liaw, J., Marshall, G., Yuan, Y., Ferreccio, C., Steinmaus, C., and Smith, A. H. (2008). Increased childhood liver cancer mortality and arsenic in drinking water in northern Chile. *Cancer Epidemiol. Biomarkers Prev.* **17**, 1982–1987.
- Ma, S., Chan, K. W., Lee, T. K., Tang, K. H., Wo, J. Y., Zheng, B. J., and Guan, X. Y. (2008). Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol. Cancer Res.* **6**, 1146–1153.
- National Science Foundation (NSF). (2005). Committee on How Toxicogenomics Could Inform Critical Issues in Carcinogenic Risk Assessment of Environmental Chemicals. In *Toxicogenomic technologies and risk assessment of environmental carcinogens: A workshop study*, pp. 1–55. National Academies Press, Washington, DC.
- Nelson, G. M., Ahlborn, G. J., Allen, J. W., Ren, H., Corton, J. C., Waalkes, M. P., Kitchin, K. T., Diwan, B. A., Knapp, G., and Delker, D. A. (2009). Transcriptional changes associated with reduced spontaneous liver tumor incidence in mice chronically exposed to high dose arsenic. *Toxicology* **266**, 6–15.
- Ouyang, W., Zhang, D., Ma, Q., Li, J., and Huang, C. (2007). Cyclooxygenase-2 induction by arsenite through the IKK β /N κ B pathway exerts an antiapoptotic effect in mouse epidermal C141 cells. *Environ. Health Perspect.* **115**, 513–518.
- Patterson, T. J., Reznikova, T. V., Phillips, M. A., and Rice, R. H. (2005). Arsenite maintains germinative state in cultured human epidermal cells. *Toxicol. Appl. Pharmacol.* **207**, 69–77.
- Patterson, T. J., and Rice, R. H. (2007). Arsenite and insulin exhibit opposing effects on epidermal growth factor receptor and keratinocyte proliferative potential. *Toxicol. Appl. Pharmacol.* **221**, 119–128.
- Pedram, A., Razandi, M., Aitkenhead, M., Hughes, C. C. W., and Levin, E. R. (2002). Integration of the non-genomic and genomic actions of estrogen; membrane-initiated signaling by steroid to transcription and cell biology. *J. Biol. Chem.* **277**, 50768–50775.
- Rice, J. M., Diwan, B. A., Donovan, P. J., and Perantoni, A. O. (1987). In *Mechanisms of Transplacental Carcinogenesis: Mutation, Oncogene Activation, and Tumor Suppression. Banbury Report 26: Developmental Toxicology: Mechanisms and Risk*, pp. 137–151. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Rountree, C. B., Ding, W., He, L., and Stiles, B. (2009). Expansion of CD133-expressing liver cancer stem cells in liver-specific phosphatase and tensin homolog deleted on chromosome 10-deleted mice. *Stem Cells* **27**, 290–299.
- Seth, P., Krop, I., Porter, D., and Polyak, K. (2002). Novel estrogen and tamoxifen induced genes identified by SAGE (Serial Analysis of Gene Expression). *Oncogene* **21**, 836–843.
- Smith, A. H., Marshall, G., Yuan, Y., Ferreccio, C., Liaw, J., von Ehrenstein, O., Steinmaus, C., Bates, M. N., and Selvin, S. (2006). Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. *Environ. Health Perspect.* **114**, 1293–1296.
- Soignet, S. L., Maslak, P., Wang, Z.-G., Jhanwar, S., Calleja, E., Dardashti, L. J., Corso, D., DeBlasio, A., Gabrilove, J., Scheinberg, D. A., et al. (1998). Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N. Engl. J. Med.* **339**, 1341–1348.
- Sullivan, J. P., Minna, J. D., and Shay, J. W. (2010). Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy. *Cancer Metastasis Rev.* **29**, 61–72.
- Tokar, E. J., Diwan, B. A., and Waalkes, M. P. (2010a). Arsenic exposure *in utero* and non-epidermal proliferative response in adulthood in Tg.AC mice. *Int. J. Toxicol.* **29**, 291–296.
- Tokar, E. J., Diwan, B. A., and Waalkes, M. P. (2010b). Arsenic exposure transforms human epithelial stem/progenitor cells into a cancer stem-like phenotype. *Environ. Health Perspect.* **118**, 108–115.
- Tokar, E. J., Qu, W., Liu, J., Liu, W., Webber, M. M., Phang, J. M., and Waalkes, M. P. (2010c). Arsenic-specific stem cell selection during malignant transformation. *J. Natl. Cancer Inst.* **102**, 638–649.
- Waalkes, M. P., Liu, J., Chen, H., Xie, Y., Achanzar, W. E., Zhou, Y.-S., Cheng, M.-L., and Diwan, B. A. (2004a). Estrogen signaling in livers of male mice with hepatocellular carcinoma induced by exposure to arsenic *in utero*. *J. Natl. Cancer Inst.* **96**, 466–474.
- Waalkes, M. P., Liu, J., and Diwan, B. A. (2007). Transplacental arsenic carcinogenesis in mice. *Toxicol. Appl. Pharmacol.* **222**, 271–280.
- Waalkes, M. P., Liu, J., Germolec, D. R., Trempus, C. S., Cannon, R. E., Tokar, E. J., Tennant, R. W., Ward, J. M., and Diwan, D. A. (2008). Arsenic exposure *in utero* exacerbates skin cancer response in adulthood with contemporaneous distortion of tumor stem cell dynamics. *Cancer Res.* **68**, 8278–8285.
- Waalkes, M. P., Liu, J., Ward, J. M., and Diwan, D. A. (2006a). Enhanced urinary bladder and liver carcinogenesis in male CD1 mice exposed to transplacental inorganic arsenic and postnatal diethylstilbestrol or tamoxifen. *Toxicol. Appl. Pharmacol.* **215**, 295–305.
- Waalkes, M. P., Liu, J., Ward, J. M., Powell, D. A., and Diwan, B. A. (2006b). Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res.* **66**, 1337–1345.
- Waalkes, M. P., Ward, J. M., and Diwan, B. A. (2004b). Induction of tumors of the liver, lung, ovary, and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. *Carcinogenesis* **25**, 133–141.
- Waalkes, M. P., Ward, J. M., Liu, J., and Diwan, B. A. (2003). Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. *Toxicol. Appl. Pharmacol.* **186**, 7–17.
- Wicha, M. S., Liu, S., and Dontu, G. (2006). Cancer stem cells: an old idea—a paradigm shift. *Cancer Res.* **66**, 1883–1890.
- Wistuba, I. I., and Gazdar, A. F. (2004). Gallbladder cancer: lessons from a rare tumour. *Nat. Rev. Cancer* **4**, 695–706.
- Yang, C., Chen, L., Li, C., Lynch, M. C., Briskin, C., and Schmidt, E. V. (2010). Cyclin D1 enhances the response to estrogen and progesterone by regulating progesterone receptor expression. *Mol. Cell Biol.* **30**, 3111–3125.
- Yuan, Y., Marshall, G., Ferreccio, C., Steinmaus, C., Liaw, J., Bates, M., and Smith, A. H. (2010). Kidney cancer mortality: fifty-year latency patterns related to arsenic exposure. *Epidemiology* **21**, 103–108.
- Zhou, Y., Eppenberger-Castori, S., Eppenberger, U., and Benz, C. C. (2005). The NF κ B pathway and endocrine-resistant breast cancer. *Endocr. Relat. Cancer* **12**, S37–S46.